Hellgren, O and Wood, Matthew J and Waldenström, J and Hasselquist, D and Ottosson, U and Stervander, M and Bensch, S (2013). *Circannual variation in blood parasitism in a sub-Saharan migrant passerine bird, the garden warbler.* Journal of Evolutionary Biology, 26 (5), 1047-1059. ISSN 1010061X

Circannual variation in blood parasitism in a sub-Saharan migrant, the garden warbler

Olof Hellgren[1*], Matthew J. Wood[1], Jonas Waldenström[2], Dennis Hasselquist[3], Ulf Ottosson[4], Staffan Bensch[3]

[1] Edward Grey Institute, Department of Zoology, South Parks Road, Oxford, OX1 3PS, United Kingdom

[2]Section for Zoonotic Ecology and Epidemiology, School of Pure and Applied Natural Sciences, Kalmar University, SE-391 82 Kalmar, Sweden

[3]Department of Animal Ecology, Ecology Building, Lund University, SE-22362 Lund, Sweden

[4]A.P. Leventis Ornithological Research Institute, University of Jos, Laminga, Jos East, Jos, Plateau, Nigeria

*Corresponding author

Keyword: *Plasmodium, Haemoproteus, Leucocytozoon, bird-migration, Sylvia borin, annual prevalence*

Abstract

Knowing the natural dynamics of pathogens in migratory birds is important in order to understand how pathogens might be transported to new geographical areas in which they may achieve transmission, and how the transmission of others might be restricted to a specific area. We studied haemosporidian blood parasites of the genera *Plasmodium, Haemoproteus* and *Leucocytozoon* in a migratory bird, the garden warbler *Sylvia borin*. Birds were sampled in spring, summer and early autumn at breeding grounds in Sweden, on migration at Capri, Italy and on arriving and departing wintering grounds in central Africa. Overall, haemosporidian prevalence was 39%, involving 24 different parasite lineages. Prevalence varied significantly over the migratory cycle, with relatively high total prevalence in the population at the breeding ground and at the onset of autumn migration, followed by marked troughs in prevalence during mid-migration both in spring and autumn. Importantly, we found that when examining circannual variation in the different lineages, clear differences in prevalence profiles emerged both between and within genera. Our results suggest that differences in prevalence profiles are the result of either different parasite transmission strategies or co-evolution between the host and the various parasite lineages. On separating parasites into common vs. rare lineages, we found that two peaks in the prevalence of rare parasites occur: on arrival at Swedish breeding grounds, and after the wintering period in Africa. Our results further stress the importance of appropriate taxonomic resolution when examining host-parasite interactions, since variation in prevalence both between and within parasite genera can show strikingly different patterns.

Introduction

For many bird species, migration is a phenomenon that either occurs at an intra-continental scale, or long-distance migration between continents where species migrate between temperate and tropical areas (Alerstam 1990). With the migration and movement of hosts comes an the increased probability for the transport of parasites to new geographical areas and hence contact with new potential host populations (Ishiguro, Takada & Masuzawa 2005; Mackenzie, Gubler & Petersen 2004; Olsen *et al.* 2006; Ricklefs *et al.* 2005; Smith *et al.* 1996; Waldenström *et al.* 2002). The transmission of parasites and diseases has traditionally been studied in systems in which the introduction event already has occurred, for example during an ongoing outbreak (Mackenzie *et al.* 2004; Ishiguro *et al.* 2005; Stenseth *et al.* 2008), or by analysing patterns on an evolutionary time scale (Hellgren *et al.* 2007). However, few studies have investigated the dynamics of pathogens in migrant bird hosts under natural conditions and over their full migratory cycles (but see some studies of avian influenza; (Munster *et al.* 2007; Latorre-Margalef *et al.* 2009). Such considerations are important, in order to understand why some pathogens might be transferred by migratory hosts to new geographical areas where they may achieve transmission, while the transmission of others may be confined to a specific area (Waldenström *et al.* 2002; Hellgren *et al.* 2007). Here we present one of the first studies that has examined the dynamics of globally transmitted pathogens (ie. avian blood parasites belonging to the genera, *Haemoproteus*, *Plasmodium* and *Leucozytozoon*) during a full migratory cycle in a long-distance migratory bird species.

Blood parasites of the genera *Haemoproteus*, *Plasmodium* and *Leucozytozoon* are a highly diverse group of vector borne blood parasites (Beadell *et al.* 2006; Bensch *et al.* 2004; Bensch *et al.* 2006; Pérez-Tris *et al.* 2007) that have a near global distribution, with the exception of Antarctica (Beadell *et al.* 2006; Hellgren *et al.* 2007; Valkiūnas 2005). It was presumed that

parasite species of the genera *Haemoproteus*, *Leucocytozoon*, and to a lesser degree *Plasmodium*, were highly host specific; i.e. that each parasite species was confined solely to a certain host species (summarized in Valkiūnas 2005), but PCR detection of parasite infections combined with molecular typing have shown that host specificity for all three genera is less strict (Bensch *et al.* 2000; Bensch *et al.* 2004; Waldenström *et al.* 2002), and there are now numerous reports of defined haematozoan parasite lineages that have been retrieved from more than one host species (Beadell *et al.* 2004; Hellgren, Pérez-Tris & Bensch 2009; Hellgren 2005; Krizanaskiene *et al.* 2006; Ricklefs, Fallon & Bermingham 2004). In extreme cases, particular parasite lineages have been found in resident birds from areas as far apart as sub-Saharan Africa and temperate regions of Scandinavia (Hellgren *et al.* 2007). Although host specificity might vary between haemosporidian genera, all three genera have been found to include parasites that have the ability of completing their life-cycle in birds from different families: the lineage BT2 (*Leucocytozoon*) has to date been found in 8 species belonging to 4 different families, the lineage WW2 (*Haemoproteus*) in 14 species belonging to 6 families and GRW4 (*Plasmodium*) in 38 species belonging to 11 different families (data retrieved 2009-02-02 from the MalAvi database; Bensch et al. 2009).

In this study, we examine circannual variation in the prevalence of 24 blood parasite lineages belonging to the genera *Plasmodium, Haemoproteus* and *Leucocytozoon* in the garden warbler *Sylvia borin*, over its full migratory cycle. The garden warblers is a long-distance migratory passerine bird species, with breeding grounds in temperate Europe and Western Asia and wintering grounds located in Western and Central Africa (Cramp 1988).

We sampled garden warblers for blood parasites at four geographical sites over the annual cycle: in chronological order; (i) early spring departure from sub-Saharan Africa in Nigeria, (ii) mid spring migration on the Mediterranean island of Capri, Italy, (iii) late spring migration on arrival in Ottenby, Sweden, (iv) at breeding grounds in Kvismare, Sweden, (v)

early autumn migration when leaving Kvismare, (vi) mid autumn migration on the island of Capri, Italy, (vii) late autumn arrival in sub-Saharan Africa in Nigeria, and (viii) early spring departure from sub-Saharan Africa in Nigeria the following calendar year. The four sampling sites show overlapping ringing recovery data for garden warblers (Figure 1), suggesting that our samples represent one interconnected population.

In this study we examined; i) how overall infection rates vary over the migratory cycle, ii) whether the different parasite genera and their component lineages showed different prevalence pattern over the migratory cycle, indicating different transmission strategies and co-evolutionary dynamics, and iii) whether being infected by a parasite that is common vs. rare in garden warblers might have different implication for the evolution of immune responses to the parasites. Therefore, we also investigated if geographical areas affected the probability of accumulating what are, for the host species, rare parasite lineages.

Method

Study species and sampling

The garden warbler is a small passerine songbird breeding across most of Europe, except the Mediterranean, and eastwards in to Russia east of the Urals (Cramp & Brooks 1992). It is primarily a woodland bird, preferring deciduous forest. It is an obligate migrant: all populations winter in sub-Saharan Africa, mainly in forested areas, from the Guinea savannah region of West and East Africa down to South Africa (Cramp & Brooks 1992). Western European populations of garden warblers winter in West Africa, and eastern birds winter in Eastern and Southern Africa. Further breeding populations have different non-breeding areas in Africa. In this study, we sampled birds breeding in Sweden and aimed to follow north European populations during migration through Europe to Nigeria in West Africa. The different populations cannot be distinguished by plumage characters, but ringing recovery

data from birds ringed in Scandinavia suggest that bird from this geographical area migrate through the Western and Central Mediterranean to the wooden savannas of eastern West Africa, including Nigeria, and then later in November – December finalise their movements by migrating to the Congo Basin (Soladoye et al.submitted, Fransson & Hall-Karlsson 2008; Bakken et al. 2006; see Figure 1). In contrast, garden warblers breeding in Western Europe, including Britain, seem to winter further west in West Africa, with six winter recoveries in Ghana and one in western Nigeria (Wernham et al. 2002).

In 2003 and 2004, we sampled garden warblers for haemosporidian parasites at Lake Kvismaren in Sweden (just after the breeding period), at Ottenby Bird Observatory, Sweden (early autumn when leaving and spring when arriving at the breeding grounds), on the island of Capri Italy (in autumn just prior to and in spring just after the migratory journey over the Mediterranean Sea) and at APLORI research institute in Jos, Nigeria (when arriving at the wintering grounds in late autumn and just before leaving the wintering grounds in early spring). For sampling dates and number of sampled birds see Table 1. Birds were caught using mist nets at all sites, and also using funnel traps at Ottenby Bird Observatory. Each bird was individually ringed, thus ensuring that no bird was sampled twice. From each individual a small blood sample was taken, under licence, from the wing by brachial venepuncture. The blood samples were stored at ambient temperatures in SET buffer (0.015M NaCl, 0.05M Tris, 0.001M EDTA, pH 8.0) during the field work, before being stored at -80°C until the DNA extraction. Total DNA was extracted using standard phenol/chloroform protocols (Sambrook, Fritch & Maniatis 1989) or amino acetate protocols (Richardson *et al.* 2001). Total extracted DNA was used for amplification of DNA from either of the genera *Plasmodium, Haemoproteus* and *Leucocytozoon.* DNA amplification was performed following the protocol and primers in Hellgren, Waldenstrom & Bensch (2004). The protocol amplifies a 480 base pair (bp) fragment of the parasite's mitochondrial cytochrome-*b* gene. Amplified PCR

products were sequenced in order to assign each parasite infection down to parasite lineage, where a single nucleotide difference is used as criterion to assign a parasite to different lineage. Two parasite lineages might differ with as little as one base pair substitution over a 480bp section of the cytochrome-*b* gene and still show different ecological properties (Pérez-Tris & Bensch 2005b; Reullier *et al.* 2006). Parasite lineages were assigned as rare if found at lower than 2% prevalence in the whole dataset.

Circannual variation in prevalence

In order to decompose circannual variation in blood parasite infection into variation between and within parasite genera over the migratory cycle, we examined parasite prevalence categorised as: i) the pooled prevalence of all observed haemosporidian infections, ii) genus specific prevalence (i.e. *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*, iii) lineage specific prevalence using the most common lineages in each genus, and iv) the prevalence rare lineages in the dataset of infected individuals (i.e. those lineages with a total prevalence less than 2%, over the whole circannual sample).

A Generalized Additive Model (GAM) is, in essence, a Generalized Linear Model in which a smoothed function of a covariate, in this case.sample date, can be considered alongside conventional linear predictors and their interactions. More complex non-linear functions are penalised such that a linear function would be retained if more parsimonious, with smoothing parameters automatically selected by penalized likelihood maximization using generalized cross validation (Wood, 2004; 2006). The smoothed term uses a cyclic spline for continuity between the end and beginning of the year (in this case leaving wintering grounds in Nigeria). We incorporated a smoothed function of sampling date as a model predictor, using binomial errors and a logit link. Patterns of prevalence were visualized by constructing predicted response GAMs of sample date on parasite infection. This approach applies the estimated

model effects (Fig 2b) to a hypothetical range of daily sampling occasions to produce the predicted response and associated confidence estimates (Fig 2c). Means are presented ±1 standard error.

In order to compare the local parasite diversity of the breeding grounds (Lake Kvismaren) and the stop-over site in West Africa, we used two datasets containing other bird species collected in the same year to generate cumulative parasite richness graphs, based on (i) parasite lineage per total sampled individuals and (ii) parasite lineage per bird species sampled. The African dataset contained 379 individuals from 59 species and the data set from the breeding ground contained 314 individuals from 31 species. The cumulative parasite richness graphs were generated by randomly generating 10000 cumulative datasets, each by randomly drawing individual or species from the existing dataset (Pérez-Tris *et al.* 2007).

Results

In total, we sampled 346 garden warblers at the different sampling sites, with an average of 43 individuals per site (Table 1). The overall prevalence of haemosporidian parasites was 39% with the highest prevalence on the breeding ground (59%) and the lowest prevalence on the autumn migration (23%, Table 1). We identified a total of 24 different parasite lineages, of which 7 were *Haemoproteus*, 9 *Plasmodium*, and 8 *Leucocytozoon* spp. lineages. Five of the 24 lineages were found on all the sampling locations (either during spring or autumn migration) (i.e. lineages, SYBOR1, WW2, SGS1, SYBOR6, SYBOR7, Figure 3). A total of 11 lineages were only found in a sample of a single individual. Twelve of the lineages have also been found infecting other species than garden warbler (Figure 3) and 12 lineages have, to date, been found exclusively in garden warblers. For Genbank accession numbers see the MalAvi database (Bensch et al. 2009).

204

205 *Circannual variation in overall prevalence*

206 A complex smoothed function of sample date was a highly significant and the most
207 parsimonious predictor of overall infections, indicating that haemosporidian infections in
208 garden warblers show significant circannual variation in overall prevalence (χ^2=18.1,
209 P=0.0032; Figure 4). Overall prevalence over the migratory cycle was at its highest on arrival
210 at the breeding grounds in Sweden, during breeding and at the onset of the southbound
211 migration. Both the spring and autumn migration showed dips in prevalence, and although the
212 prevalence on the wintering grounds was somewhat higher than during migration, it was still
213 lower than on the breeding grounds (Figure 3; supporting Figure 2a).

214

215 *Disentangling variation in prevalence between genera*

216 Two of the three parasite genera were predicted by smoothed sampling date: both
217 *Haemoproteus* (χ^2=38.2, P<0.0001) and *Plasmodium* (χ^2=7.58, P=0.038) showed significant
218 circannual variation in prevalence, whereas *Leucocytozoon* did not (χ^2=0.095, P=0.76). The
219 circannual prevalence profile of *Haemoproteus* infection (Figure 3a) showed a similar pattern
220 to the overall prevalence although at a slightly lower prevalence. The annual patterns of
221 *Plasmodium* and *Leucocytozoon,* however, show strikingly different patterns. *Plasmodium*
222 showed the lowest prevalence during breeding and the onset of migration and then a slight
223 increase in prevalence when arriving and leaving the wintering grounds. The overall
224 *Leucocytozoon* prevalence was at an almost constant level all over the migratory cycle.

225

226 *Disentangling variation within genera*

227 Lineages belonging to the same genera can have widely different prevalence profiles in a
228 population over a certain year (Cosgrove *et al.* 2008). We examined the most prevalent

lineages in each genus in order to disentangle lineage-specific transmission patterns and co-evolutionary traits. The two most common parasite lineages of *Haemoproteus* (WW2 and SYBOR1) showed very different annual patterns. WW2 showed a highly significant circannual variation (χ^2=25.6, P<0.0001), with high prevalence during breeding and the onset of migration, and absence during the wintering period (Figure 5b). The other widely prevalent *Haemoproteus* lineage, SYBOR1, did not show any significant circannual variation in prevalence (χ^2=7.21, P=0.11), prevalence instead being more evenly spread over the migratory cycle with a small increase in prevalence in winter (Figure 5c). The higher prevalence of *Plasmodium* spp. in winter (Figure 6a) was not explained by variation in the most common *Plasmodium* lineage, SGS1, which showed relatively flat prevalence over the whole year (Figure 6b). While pooled *Leucocytozoon* infections showed no circannual variation in prevalence, examining the two most common *Leucocytozoon* lineages revealed contrasting patterns: BT2 showed significant circannual variation in prevalence (χ^2=11.8, P=0.020), with a bimodal distribution with one peak in late spring migration and another during early autumn migration (Figure 7b). The lineage SYBOR7 showed a more evenly distributed prevalence over the migratory cycle, although its circannual pattern only approached statistical significance (χ^2=5.78, P=0.062; Figure 7c).

Rare parasite lineages and lineage diversity

17 out of 18 rare lineages (i.e. lineages at <2% prevalence in the total sample) occurred only on arrival on breeding grounds or after the birds have visited the wintering grounds (Figure 3). When analysing the occurrence of rare lineages over the migratory cycle, the highest probability of finding a "rare" lineage occurred when garden warblers were sampled in Africa or when they arrived on the Swedish breeding grounds (χ^2=21.42, P=0.006, Figure 8). Seven of these rare lineages are known to be transmitted in Europe, because the lineages have either

been found in juvenile migrants before autumn migration, or in a resident European bird species. Three other of the rare lineages have been found in African resident bird species, thus confirming transmission in Africa (Figure 3).

Discussion

We have shown that the prevalence of haemosporidian blood parasites in a migratory bird species varies significantly over the annual cycle, with high overall prevalence in the population on the breeding grounds and at the onset of autumn migration, followed by marked decreases in prevalence during mid-migration, both in spring and autumn. When disentangling the patterns in prevalence both between and within parasite genera, clear differences emerged. Our results strongly suggest that the differences in prevalence profiles are a result of either different parasite transmission strategies or co-evolution between the host and the different parasite lineages. Therefore, we stress the importance of considering that a range of different host-parasite interactions might underly apparent variation in overall parasite prevalence, and that such taxonomic resolution should be taken into account when examining parasite-induced fitness effects. For example, the *Haemoproteus* parasite WW2 might be postulated not to have any detrimental effect on migration, as it was absent in host blood during the migration period. In contrast, the related *Haemoproteus* parasite SYBOR1 might potentially have detrimental effects, as it was found most frequently in the blood of garden warblers during migration.

The absence of a parasite in the blood might either be due to that the individual is not infected, the parasite is dormant and found in tissues and not the bloodstream (Valkiūnas 2005), or that is occurs in at such low intensities in the blood that it is not detectable by PCR screening. If the parasite is found in the blood of the host, it is, in the case of *Haemoproteus* and *Leucocytozoon,* always as gametocytes, i.e. at the final (sexual) transmission stage of the

parasite (Valkiūnas 2005); whereas in the case of *Plasmodium,* the blood can also include asexual reproduction stages of the parasites. When present in the blood, haemosporidian parasites infect blood cells which are lysed to different degrees, potentially causing different degrees of anaemia (van Riper and Atkinson 1991). Thus, there might be a trade-off for the parasite, either (i) to be in the bloodstream and potentially harm the host but also being available to be transmitted by a vector, or (ii) to stay dormant in host tissues; probably causing less severe fitness effects but thereby loosing the potential to be transmitted. The outcome of this trade-off for the parasite is likely to be mainly influenced by the probability of parasite transmission, which in turn is influenced by the abundance of compatible vectors and the effects the infection have on the host.

When investigating parasite prevalence in correlative studies of wild populations, it is difficult to identify the processes behind the observable patterns. For example, low prevalence could result from (i) the absence of infected individuals due to high parasite-induced mortality of the hosts, (ii) the parasite's strategy not to be in the blood stream at a given point in the migratory cycle, or that (iii) individuals either having not been exposed to the parasite or having recovered from the infection. Similarly, high prevalence can be caused by several mutually operating processes such as; (i) an active strategy of the parasite to be out in the bloodstream to enable transmission, (ii) physical stress of the host that suppresses its immune function, and (iii) a high exposure of the host to the parasite in question. We will discuss our observed prevalence pattern in the light of these scenarios.

Overall prevalence

Being a migrant bird might not only include the cost of considerable physiological stress during migration, but might also include exposure to avian blood parasites over the whole calendar year as compared to resident bird species, such as in tropical areas where parasites

tend to be more abundant (Møller & Erritzøe 1998, Hasselquist 2007). In the case of the garden warbler, the pooled prevalence patterns reveal that a proportion of the population carry active infections by some kind of blood parasite throughout the whole annual cycle (Figure 4), whereas in resident bird species of the temperate region parasites disappear from the blood stream during the cold periods of the year (Cosgrove *et al.* 2008). The marked differences between the species could stem from either of two differences. On one hand, the lack of parasites during winter in the resident bird species could be a result of clearance of the infection during the winter and then becoming reinfected during spring: the presence of infection in the garden warblers during winter would then be infections of parasites that have latent infections. On the other hand, the strategy of parasites of resident species may involve leaving the blood stream for dormancy in the tissues during winter, due to the absence of vectors and thus no possibilities of transmission, and subsequently relapsing in spring when transmission becomes possible again with the return of vectors. In the garden warbler, the occurrence of winter infections could thus be due to some parasites having different transmission periods to match patterns of vector abundance at each site.

The overall prevalence pattern showed a peak during the breeding period and at the arrival in West Africa, with prevalence troughs during spring and autumn migration periods. During migrations, parasites might stay dormant or at levels of parasitaemia below detection for several reasons. First, suitable vectors might be absent from stopover sites, and once the parasites finally have matured in the vector the majority of hosts might already have passed through. Secondly, the migration in it self might reduce the survival of the host, and if the parasite is patent in the bloodstream the survival of the host might be further reduced, thus also reducing the survival of the parasite without the gain of potential transmission. However a study of redwings *Turdus iliacus* showed a contrasting pattern, with experimentally induced Zugunruhe (migratory restlessness) resulting in relapses of dormant infections of *Borellia*

329 *garnii,* a spirochaete bacterium (Gylfe *et al.* 2000). One possibility for the contrasting
330 patterns between haemopsporidia and *Borellia* could be due to different effects on host
331 survival leading to different evolutionary strategies,or that *Borrelia* also shares hosts across
332 species (i.e. mammals as well as migratory birds) that do not migrate.
333 An alternative explanation for the overall lower prevalence during migration might be a
334 consequence of reduced survival caused by the parasite, such that the host with detectable
335 parasitaemia suffers from high mortality during demanding migratory journeys, such as the
336 crossing of Sahara or the Mediterranean, compared to individuals with low levels of infection.
337 The high prevalence when arriving to the breeding grounds at the final stage of their
338 northward spring migration would then result from relapses in individuals that were able to
339 keep the intensity of the infection at a low level during migration (Figure 4).
340
341 *Lineage specific prevalence patterns*
342 When decomposing total haemosporidian prevalence into genus specific prevalence, we
343 observed that the mid migration troughs in prevalence are mainly due to circannual variation
344 in *Haemoproteus* lineages (Figure 4, 4a), and that the wintering peak is to some extent
345 augmented by *Plasmodium* infections. When further dividing the *Haemoproteus* lineages into
346 the two most common lineages, we found two totally different patterns which shed light on
347 the observed mid migration troughs in prevalence.
348 The increase in prevalence of the WW2 lineage starts already when birds are arriving to the
349 breeding grounds in spring and the high prevalence lasts until they are leaving the breeding
350 ground in northern Europe in autumn. Moreover, we know that this lineage is transmitted in
351 Europe whereas we have no indication of transmission in Africa. The lineage is then absent in
352 the population during the mid-migration period as well as on the wintering grounds. This
353 could be a consequence either of the parasite's dormancy in internal host organs, or the

354 impossibility of transmission in Africa due to vector availability or climate, for example.
355 However, we cannot exclude the possibility of host recovery from WW2 infections in late
356 summer. However, based on our data, it is more likely that the parasite is dormant during
357 autumn and winter, because we find it in the blood of migrants at the arrival on the breeding
358 grounds (found in two birds in late May). For these birds to have a detectable infection, the
359 biting midge that infected them must have taken its blood meal in late April, when passing
360 stop-over sites in southern Europe.
361 The second lineage SYBOR1 is found throughout the year (Figure 3) with a prevalence peak
362 on the wintering grounds (Figure 5c). This suggests either that transmission does occur in
363 both the breeding and the wintering areas, or, if no circannual transmission is possible, that
364 SYBOR1 is not yet adapted to the migratory host. Tropically transmitted haemosporidian
365 parasites do occur in the bloodstream during summer in tropical migrants without
366 transmission having taken place (Bensch *et al.* 2006, Hellgren *et al.* 2007).
367 Pooling the prevalence of parasites with different transmission strategies may result in
368 spurious circannual patterns in prevalence during migration. For example, in the case of our
369 garden warbler study, a trough in total haemosporidian prevalence during autumn migration
370 may constitute a break point where one lineage (WW2) has dropped in prevalence perhaps
371 because of the difficulty of transmission in Africa, and another lineage (SYBOR1) is just
372 about to rise in prevalence when the birds arrive at their wintering grounds where this lineage
373 has its highest prevalence (Figure 5a-c). Hence, the complex interaction of a rather large
374 number of parasite lineages within each haemosporidian genera, with different prevalence
375 patterns over the annual cycle, makes it dangerous to interpret prevalence patterns based on
376 lineages pooled within genera. Our data strongly implies that in order to understand the
377 interactions between blood parasites and their bird hosts, it is essential to identify parasite
378 lineages and to monitor their occurrence and effects on hosts separately.

379 The *Leucocytozoon* spp. prevalence remained stable and low over the whole annual cycle in
380 the garden warbler. However, a closer inspection of the two most common lineages reveals
381 that, in fact, circannual patterns also exist for *Leucotyzoon*. The BT2 lineage had a bimodal
382 shape, with peaks when the birds arrived and left the breeding grounds (Figure 7b). For this
383 lineage we also have confirmed that transmission occurs in Northern Europe, indicating that
384 this parasite is adapted to circulate in peripheral blood when they have the possibility of being
385 transmitted. A contrasting temporal pattern was seen for SYBOR7, a lineage that occurred at
386 all sampling locations, with a slight elevation in prevalence during the non-breeding period in
387 Africa (Figure 6a-c). The differences between these two *Leucocytozoon* lineages strongly
388 suggest that the parasites have adopted different transmission strategies.
389 The prevalence of *Plasmodium* spp. was comprised of many rare lineages, most of them
390 detected mainly during the non-breeding period (Figure 6). SGS1, the most common
391 *Plasmodium* lineage, had a prevalence curve which was apparently independent of time and
392 location. This corroborates earlier findings which have found that the SGS1 lineage is one of
393 very few lineages that can be transmitted both in Africa and Europe (Hellgren et al. 2007).
394
395 *Transmission of rare parasite lineages*
396 When screening a passerine bird species for avian blood parasites, a common finding is that
397 the parasite community within that host species often is comprised of a few common lineages
398 followed by a tail distribution of rare parasite lineages (found in a few or a single host
399 individuals). This pattern has been found also in other well-sampled European passerine bird
400 species, such as blackcaps (Pérez-Tris & Bensch 2005), great reed warblers *Acrocephalus*
401 *arundinaceus* (Bensch *et al.* 2006) and house sparrows *Passer domesticus* (Bonneaud *et al.*
402 2006). This pattern was also apparent in the garden warbler (Figure 3). Importantly, the tail of
403 rare lineages comprised 25% of all infections (defining uncommon lineages as those

404 constituting ≤ 2% of total prevalence). For the host, however, rare parasite lineages might also
405 have important evolutionary implications. When hosts are exposed to common parasites this
406 should result in co-evolution between parasites and the host, as every evolutionary change in
407 the host or the parasite that increases host survival would also be beneficial for their offspring,
408 because they are likely to be exposed subsequently to the same common parasite lineages.
409 However, with the uncommon lineages the scenario might be different, as even though the
410 chance of being exposed to and infected by an uncommon lineage is fairly high, the
411 probability of the offspring being infected by the same lineage is low. This scenario may have
412 implications for the evolution of the immune system, in terms of having a broad defence
413 against a wide array of parasites or an immune system adapted to some frequently
414 encountered lineages. In our case the uncommon lineages were found predominantly in
415 samples from the non-breeding area, likely reflecting increased parasite diversity in the
416 African bird community (Møller & Erritzøe 1998, Hasselquist 2007, see also Figure 9). If so,
417 this would mean that by being a migrant, birds not only increase the time over which they are
418 exposed to parasites (as compared to resident birds in temperate regions that lack parasite
419 transmission during autumn and winter (Cosgrove *et al.* 2008)), but they are also exposed to a
420 higher diversity of parasites by visiting areas with totally different bird communities and their
421 accompanying parasites. Hence, this then constitutes a 'cost of migration' (Waldenström *et al.*
422 2002) with important implications. For example, being a migrant bird would mean quite
423 different demands on the immune system being exposed to a more diverse parasite fauna, as
424 compared to resident bird species that might be able to adapt to a more stable and
425 homogenous parasite fauna (Hasselquist 2007).
426
427 *Concluding remarks*

428 This is one of the first studies that to follow the parasitism in a migratory passerine bird
429 species over the whole annual cycle. By doing, so we have highlighted that the transmission
430 strategies of a parasite might have strong effects on its potential to be transported to new
431 areas. For example, a parasite adapted to transmission in Europe during summer and which is
432 not present in the blood during migration would have very low chances of infecting African
433 bird species. We have further shown that related parasites can have very different circannual
434 prevalence patterns in the same host species. Of importance for future studies to gain a more
435 fully understanding how well the parasites are adapted to the annual migration of its host, is to
436 investigate how the virulence i.e. the intensity of the parasites, varies during the migration.
437

438 **Acknowledgement**

439 We would like to thank A.P. Leventis Ornithological Research Institute in Jos, Nigeria,
440 Ottenby Bird Observatory in Sweden and the Villa San Michele on Capri, both of which
441 kindly provided accommodation, logistic support and field assistance during our fieldwork.
442 Martin Stervander helped visualise ringing recoveries. Financial support was received from
443 the Swedish Research Council (to OH, DH, SB), the Swedish Research Council for
444 Environmental, Agricultural Science and Spatial Planning (to DH) and the UK Natural
445 Environment Research Council (to MJW). This is contribution no. XX from A.P. Leventis
446 Ornithological Research Institute and no XXX from Ottenby Bird Observatory. XX Kvismare
447 B:O.
448
449

450 References
451

452 Alerstam,T. (1990) *Bird migration*. Cambridge University Press, Cambridge.

Bakken, V., Runde, O. & Tjorve, E. 2006. norsk ringmerkingsatlas. Vol 2. Stavanger Museum, Stavanger

Beadell,J.S., Gering,E., Austin,J., Dumbacher,J.P., Peirce,M.A., Pratt,T.K., Atkinson,C.T. & Fleischer,R.C. (2004) Prevalence and differential host-specificity of two avian blood parasite genera in the Australo-Papuan region. *Molecular Ecology*, **13,** 3829-3844.

Beadell,J.S., Ishtiaq,F., Covas,R., Melo,M., Warren,B.H., Atkinson,C.T., Bensch,S., Graves,G.R., Jhala,Y.V., Peirce,M.A., Rahmani,A.R., Fonseca,D.M. & Fleischer,R.C. (2006) Global phylogeographic limits of Hawaii's avian malaria. *Proceedings of the Royal Society B-Biological Sciences*, **273,** 2935-2944.

Bensch,S., Hellgren,O. & Pérez-Tris, J. (2009) MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages**.** *Molecular Ecology Resources*, **9**, 1353-1358.

Bensch,S., Pérez-Tris,J., Waldenstrom,J. & Hellgren,O. (2004) Linkage between nuclear and mitochondrial DNA sequences in avian malaria parasites: Multiple cases of cryptic speciation? *Evolution*, **58,** 1617-1621.

Bensch,S., Stjernman,M., Hasselquist,D., Ostman,O., Hansson,B., Westerdahl,H. & Pinheiro,R.T. (2000) Host specificity in avian blood parasites: a study of Plasmodium and Haemoproteus mitochondrial DNA amplified from birds. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **267,** 1583-1589.

Bensch,S., Waldenström,J., Jonzen,N., Westerdahl,H., Hansson,B., Sejberg,D. & Hasselquist,D. (2007) Temporal dynamics and diversity of avian malaria parasites in a single host species. *Journal of Animal Ecology* **76**:112–122.

Bonneaud,C., Pérez-Tris,J., Federici,P., Chastel,O. & Sorci,G. (2006) Major histocompatibility alleles associated with local resistance to malaria in a passerine. *Evolution*, **60,** 383-389.

Cosgrove,C.L., Wood,M.J., Day,K.P. & Sheldon,B.C. (2008) Seasonal variation in Plasmodium prevalence in a population of blue tits Cyanistes caeruleus. *Journal of Animal Ecology*, **77,** 540-548.

Cramp,S. (1988) Handbook of the birds of Europe the Middle east and North Africa: The birds of Western Palearctic. Oxford University Press, Oxford.

Cramp,S. & Brooks,D.J. (1992) *Handbook of the birds of Europe, the Middle East and North Africa the birds of the Western Palearctic* Oxford University Press, Oxford.

Fransson, T. & Hall-Karlsson, S. 2008. Svensk ringmärkningsatlas. Vol. 3. Stockholm

Gylfe,Å., Bergström,S., Lundström,J. & Olsen,B. (2000) Reactivation of Borrelia infection in birds. *Nature,* **403**, 724-725.

Hasselquist, D. (2007) Comparative immunoecology in birds: hypotheses and tests. *Journal of Ornithology***, 148** (Suppl. 2), 571-582.

Hellgren,O., Pérez-Tris,J. & Bensch,S. A jack of all trades and still a master of some-associations between host range and prevalence in avian malaria and related blood parasites. Ecology . 2009.
Ref Type: In Press

Hellgren,O. (2005) The occurrence of haemosporidian parasites in the Fennoscandian bluethroat (Luscinia svecica) population. *Journal of Ornithology*, **146,** 55-60.

Hellgren,O., Waldenstrom,J. & Bensch,S. (2004) A new PCR assay for simultaneous studies of Leucocytozoon, Plasmodium, and Haemoproteus from avian blood. *Journal of parasitology*, **90,** 797-802.

Hellgren,O., Waldenstrom,J., Pérez-Tris,J., Szollosi,E., Hasselquist,D., Krizanauskiene,A., Ottosson,U. & Bensch,S. (2007) Detecting shifts of transmission areas in avian blood parasites - a phylogenetic approach. *Molecular Ecology*, **16,** 1281-1290.

Ishiguro,F., Takada,N. & Masuzawa,T. (2005) Molecular evidence of the dispersal of Lyme disease Borrelia from the Asian continent to Japan via migratory birds. *Japanese Journal of Infectious Diseases*, **58,** 184-186.

Krizanaskiene,A., Hellgren,O., Kosarev,V., Sokolov,L., Bensch,S. & Valkiūnas,G. (2006) Variation in host specificity between species of avian hemosporidian parasites: Evidence from parasite morphology and cytochrome B gene sequences. *Journal of parasitology*, **92,** 1319-1324.

Latorre-Margalef,N., Gunnarsson,G., Munster,V.J., Fouchier,R.A.M., Osterhaus,A.D.M.E., Elmberg,J., Olsen,B., Wallensten,A., Haemig,P.D., Fransson,T., Brudin,L. & Waldenstr+Âm,J. (2009) Effects of influenza A virus infection on migrating mallard ducks. *Proceedings of the Royal Society B: Biological Sciences*, **276,** 1029-1036.

Mackenzie,J.S., Gubler,D.J. & Petersen,L.R. (2004) Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nature Medicine*, **10,** S98-S109.

Munster,V.J., Baas,C., Lexmond,P., Waldenstrom,J., Wallensten,A., Fransson,T., Rimmelzwaan,G.F., Beyer,W.E.P., Schutten,M., Olsen,B., Osterhaus,A.D.M.E. & Fouchier,R.A.M. (2007) Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. *Plos Pathogens*, **3,** 630-638.

Møller A.P, Erritzøe, J. (1998) Host immune defence and migration in birds. *Evoloutionary Ecolology,* **12**, 945-953.

Olsen,B., Munster,V.J., Wallensten,A., Waldenstrom,J., Osterhaus,A.D.M.E. & Fouchier,R.A.M. (2006) Global patterns of influenza A virus in wild birds. *Science*, **312,** 384-388.
Pérez-Tris,J. & Bensch,S. (2005) Dispersal increases local transmission of avian malarial parasites. *Ecology Letters*, **8,** 838-845.

Pérez-Tris,J., Hellgren,O., Krizanauskiene,A., Waldenström,J., Secondi,J., Bonneaud,C., Fjeldså,J., Hasselquist,D. & Bensch,S. (2007)Within-host speciation of malaria parasites. PLoS One **2**(2):e235. [doi: 10.1371/journal.pone.0000235]

Reullier,J., Pérez-Tris,J., Bensch,S. & Secondi,J. (2006) Diversity, distribution and exchange of blood parasites meeting at an avian moving contact zone. *Molecular Ecology*, **15,** 753-763.

Richardson,D.S., Jury,F.L., Blaakmeer,K., Komdeur,J. & Burke,T. (2001) Parentage assignment and extra-group paternity in a cooperative breeder: the Seychelles warbler (Acrocephalus sechellensis). *Molecular Ecology*, **10,** 2263-2273.

Ricklefs,R.E., Fallon,S.M. & Bermingham,E. (2004) Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. *Systematic Biology*, **53,** 111-119.

Ricklefs,R.E., Fallon,S.M., Latta,S.C., Swanson,B.L. & Bermingham,E. (2005) Migrants and Their Parasites; A bridge between two worlds. *Birds of Two Worlds; the ecology and evolution of migration* (eds R. Greenberg & P. P. Marra), pp. 210-221. The Johns Hopkins University Press, Baltimore.

Sambrook,J., Fritch,F.J. & Maniatis,T. (1989) *Molecular clooning, a labaratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Soladoye,B.I., Ottosson,U., Yahkat,B., Helseth,A., Hulme,M.F., Stevens,M. & Waldenström, J. From the savanna to the final wintering ground – the non- breeding movements of the Garden Warbler *Sylvia borin* in Nigeria (Submitted).

Smith,R.P., Rand,P.W., Lacombe,E.H., Morris,S.R., Holmes,D.W. & Caporale,D.A. (1996) Role of bird migration in the long-distance dispersal of Ixodes dammini, the vector of Lyme disease. *Journal of Infectious Diseases*, **174,** 221-224.

Stenseth,N.C., Atshabar,B.B., Begon,M., Belmain,S.R., Bertherat,E., Carniel,E., Gage,K.L., Leirs,H. & Rahalison,L. (2008) Plague: Past, present, and future. *Plos Medicine*, **5,** 9-13.

Valkiūnas,G. (2005) *Avian malaria parasites and other haemosporidia*. CRC, Boca Raton, Florida.

Waldenström,J., Bensch,S., Kiboi,S., Hasselquist,D. & Ottosson,U. (2002) Cross-species infection of blood parasites between resident and migratory songbirds in Africa. *Molecular Ecology*, **11,** 1545-1554.

Wood, S.N. (2004) Stable and efficient multiple smoothing parameter estimation for generalized additive models. *Journal of the American Statistical Association* **99**:673-686

Wood, S.N. (2006) *Generalized Additive Models: An Introduction with R*. Chapman and Hall/CRC

Wood, M.J., Cosgrove, C.L., Wilkin, T.A., Knowles, S.C.L., Day, K.P. & Sheldon, B.C. (2007) Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, *Cyanistes caeruleus*. *Molecular Ecology*, **16,** 3263-3273.

Wernham, C.V., Toms, M.P., Marchant, J.H., Clark, J.A., Siriwardena, G.M. & Baille, S.R. (eds) 2002. *The Migration Atlas: Movement of the Birds of Britain and Ireland*. T. & A.D. Poyser, London

Figure 1. Ringing recoveries of garden warblers. The insert map shows the location of the four sampling sites in this study, the main map shows the locations of garden warblers that were subsequently recaptured elsewhere. The symbols used in the main map of recovery sites match the site where the bird was initially ringed.

Figure 2. Construction of Generalized Additive Models

In this case, raw prevalence data (Fig. 7a) are summarized by a smoothed model using penalised least squares regression (Fig 7b: estimated model effect plotted ±1s.e.). The model may be visualised by examining the fitted relationship with a predictor, in this case calendar (January) date; the predicted response model (Fig 7c), which is presented subsequently to visualise circannual variation in prevalence (model fit ±1 s.e.). See Methods for further details.

Figure 3. N-J tree of all found parasite lineages in the garden warblers.

Bars represent total prevalence for each of the lineages, coloured boxes show sampling sites at which each of the lineages were found in this study. Transmission areas for a parasite lineage are determined by the presence of the lineage in either (i) a juvenile bird before migration, or (ii) in a resident bird species in either Africa or Europe. The number of additional host species in which each lineage has been found is displayed in the right column. For Genbank accession numbers see MalAvi database (Bensch et al. 2009).

Figure 4. Circannual variation in haemosporidian prevalence between genera.

Fitted prevalence functions for; pooled infections, *Leucocytozoon* infections, *Haemoproteus* infections, and *Plasmodium* infections.

Figure 5. Circannual variation within genera: *Haemoproteus*

Fitted prevalence functions for (a) pooled *Haemoproteus* infections, (b) *Haemoproteus* lineage WW2, (c) *Haemoproteus* lineage SYBOR1. Smoothed functions are plotted ±1 s.e.

Figure 6. Circannual variation within genera: *Plasmodium*

Fitted prevalence functions for (a) pooled *Plasmodium* infections, (b) *Plasmodium* lineage SGS1. Smoothed functions are plotted ±1 s.e.

Figure 7. Circannual variation within genera: *Leucocytozoon*

Fitted prevalence functions for (a) pooled *Leucocytozoon* infections, (b) *Leucocytozoon* lineage BT2, and (c) *Leucocytozoon* lineage SYBOR7. Smoothed functions are plotted ±1 s.e.

Figure 8. Circannual variation in the prevalence of rare parasite lineages.

Rare parasite lineages were defined as those with less than 2% prevalence. A fitted prevalence function was estimated only among infected individuals.

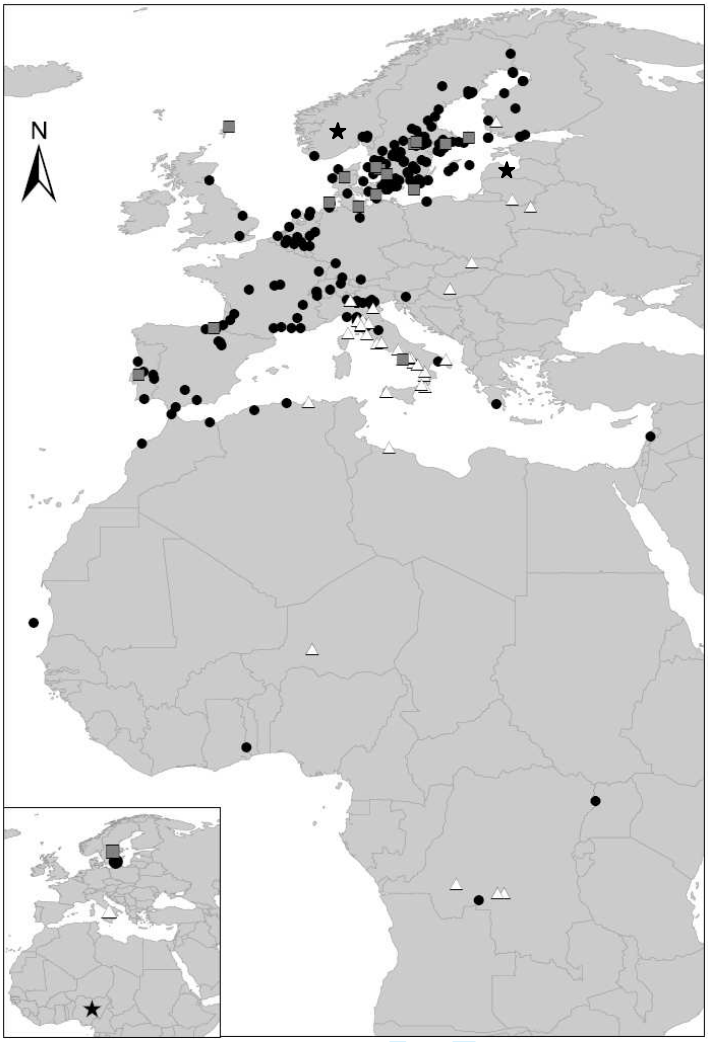
Figure 9. Parasite species richness in Nigeria and Sweden

Cumulative richness graphs with 95% C.I. (based on 1000 Monte-Carlo simulations) of parasite lineages (i.e. *Plasmodium, Leucocytozoon* and *Haemoproteus* spp) found in two different bird communities. Red slope; Jos, Nigeria. Blue Slope; Kvismare Sweden. Both sampling periods coincide with periods in which garden warblers were sampled at the two sites. Graphs based on (a) individual sampled bird, (b) based on sampled bird species.

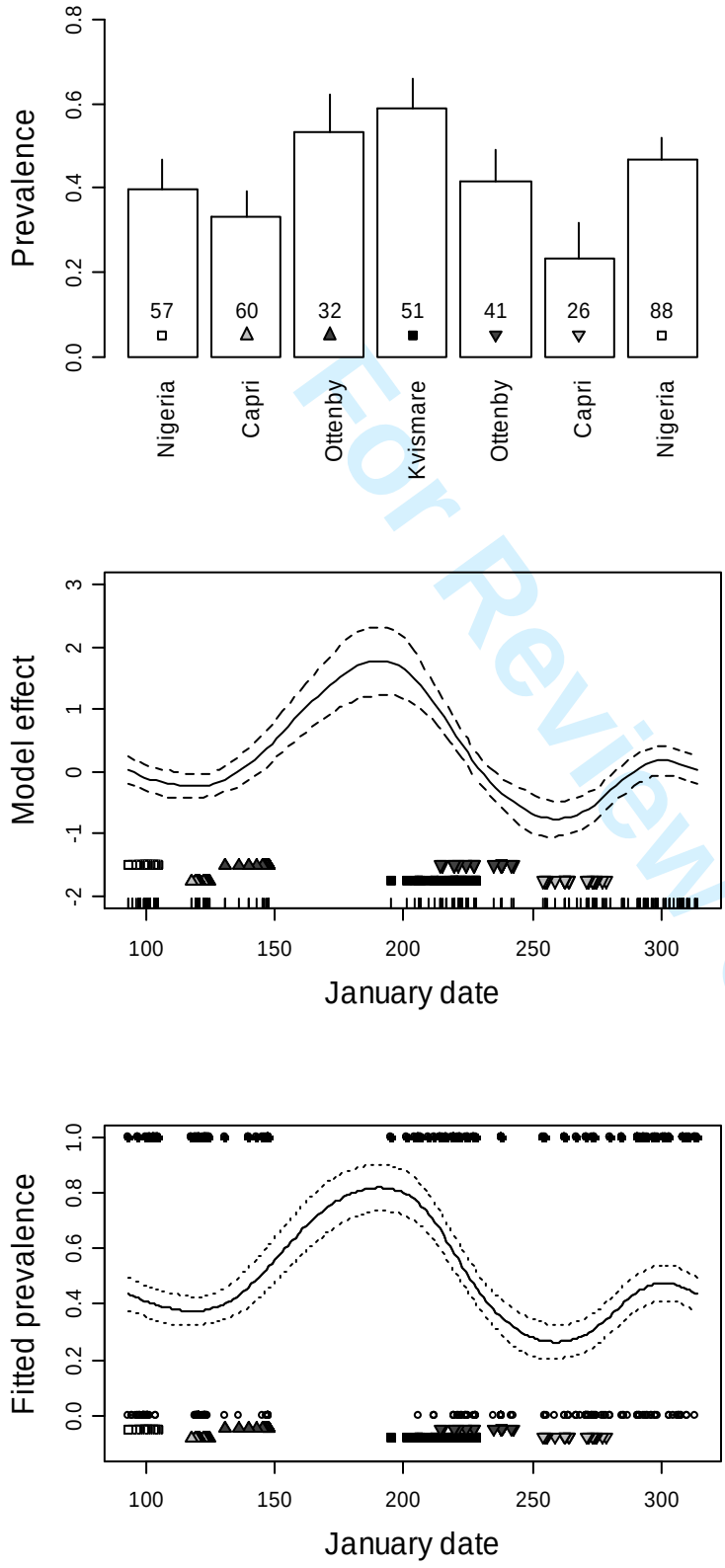
Table 1. Sampling sites, dates of sampling and number of sampled garden warblers. Site specific prevalence is shown for all haemopsporidian parasites pooled (i.e. *Haemoproteus*, *Plasmodium* and *Leucocytozoon* spp) as well as genus specific prevalence for the different sites

Migratory phase	**Place**	**Year**	**Date**	**N**	**Prevalence**				**Number of lineages**			
					Any	***Haem.***	***Plas.***	***Leuco.***	***Any***	***Haem.***	***Plas.***	***Leuco.***
Arrival wintering ground, autumn migration	Nigeria, Jos	2003	18/10-7/11	57	0.53	0.26	0.14	0.16	11	1	7	3
Leaving wintering ground, spring migration	Nigeria, Jos	2004	2/4-14/4	48	0.40	0.06	0.13	0.21	9	1	4	4
Spring migration	Italy, Capri	2004	27/4-4/5	60	0.33	0.10	0.05	0.18	8	1	3	4
Arriving breeding ground, spring migration	Sweden, Ottenby	2004	10/5-27/5	32	0.53	0.25	0.06	0.31	8	4	2	2
Breeding ground	Sweden, Kvismare	2004	13/7-15/8	51	0.59	0.57	0.02	0.08	7	4	1	2
Leaving breeding ground, autumn migration	Sweden, Ottenby	2004	1/8-25/8	41	0.41	0.10	0.00	0.34	6	3	0	3
Autumn migration	Italy, Capri	2004	10/9-5/10	26	0.23	0.08	0.08	0.08	5	2	2	1
Arrival wintering ground, autumn migration	Nigeria, Jos	2004	23/9-27/10	31	0.35	0.19	0.13	0.16	10	3	4	3
TOTAL				***346***	***0.39***	***0.18***	***0.06***	***0.19***	***24***	***7***	***9***	***8***

Figure 1

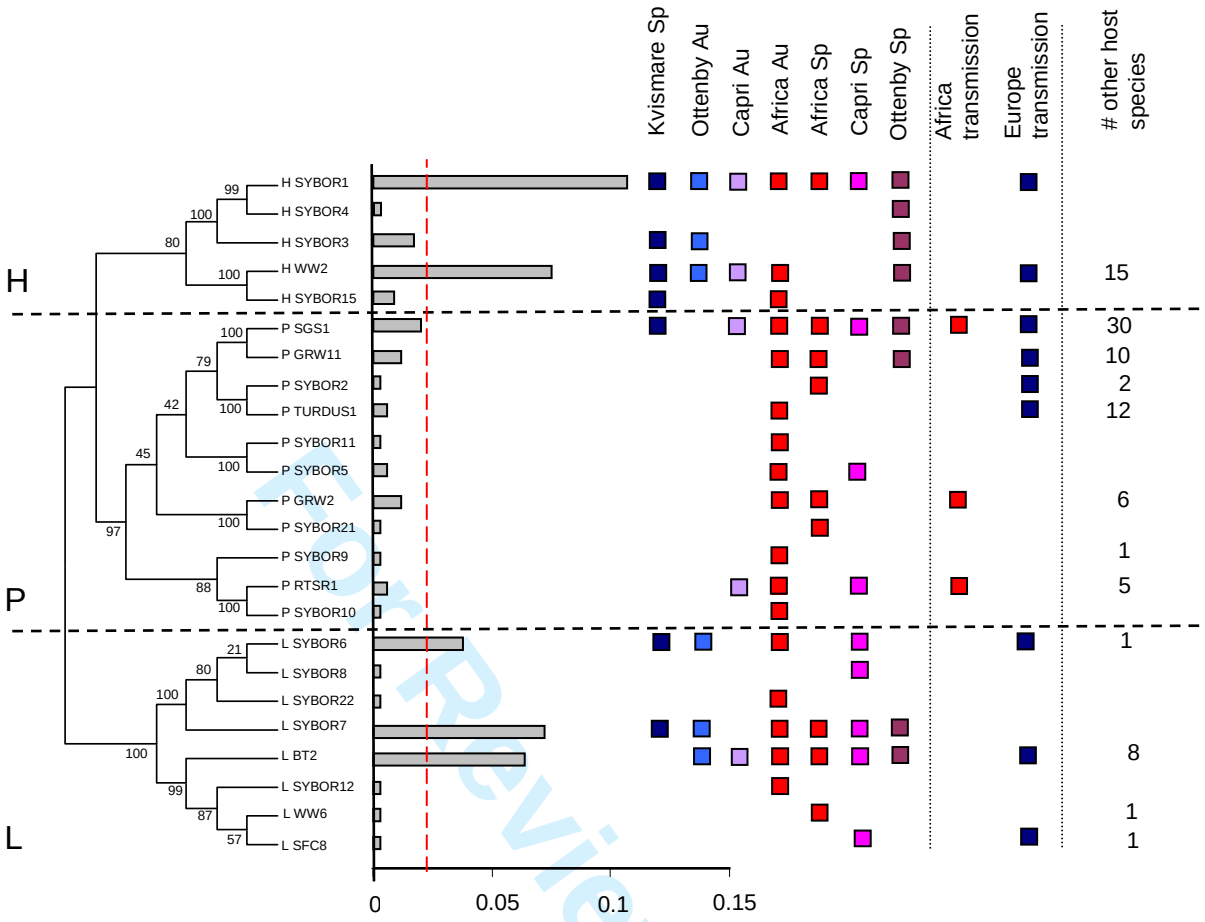


27 FIGURE 2.

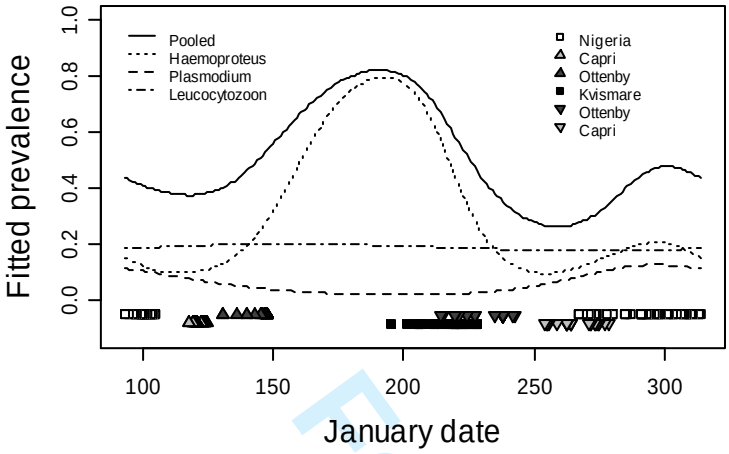


28

29 FIGURE 3.

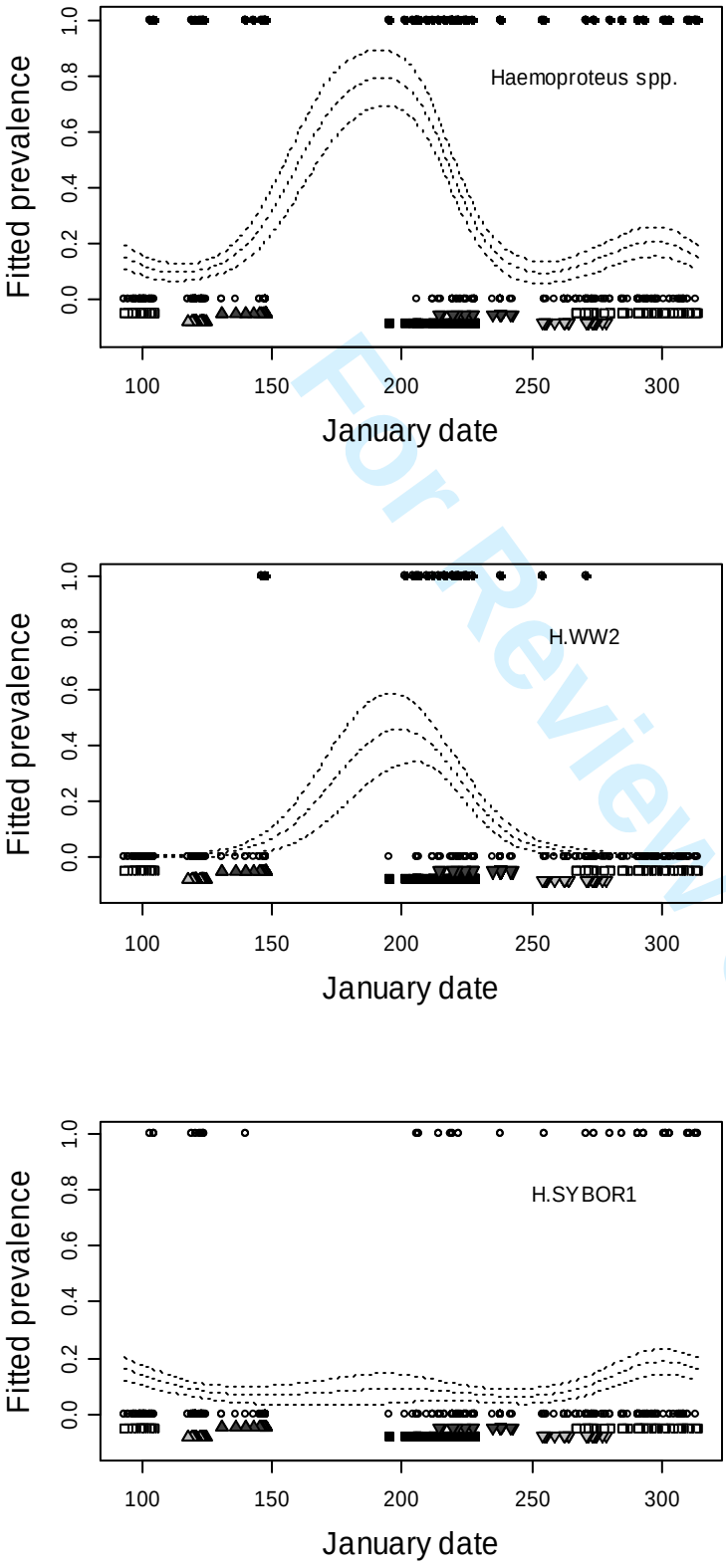


30 FIGURE 4.



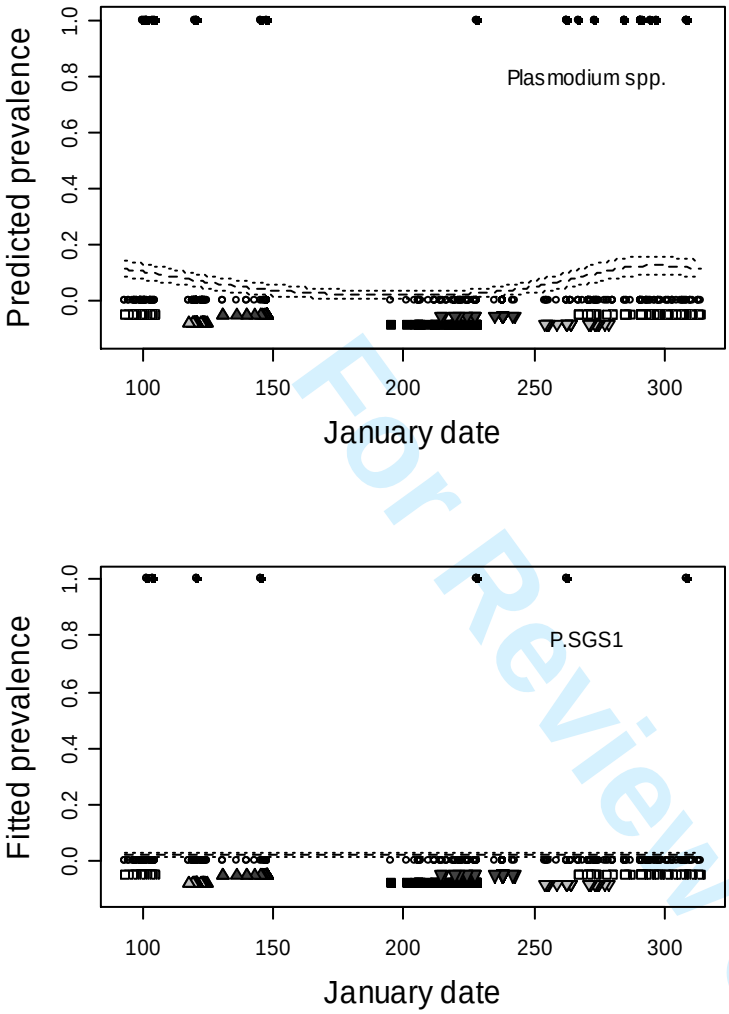
31

32 FIGURE 5a-c.
33



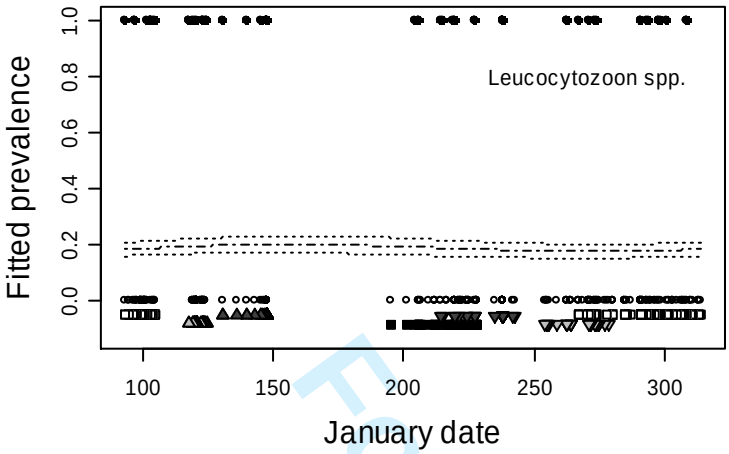
34

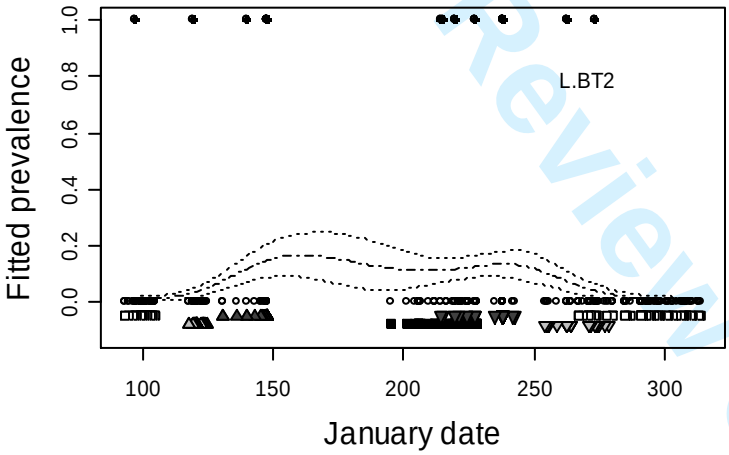
35 FIGURE 6a-b.
36

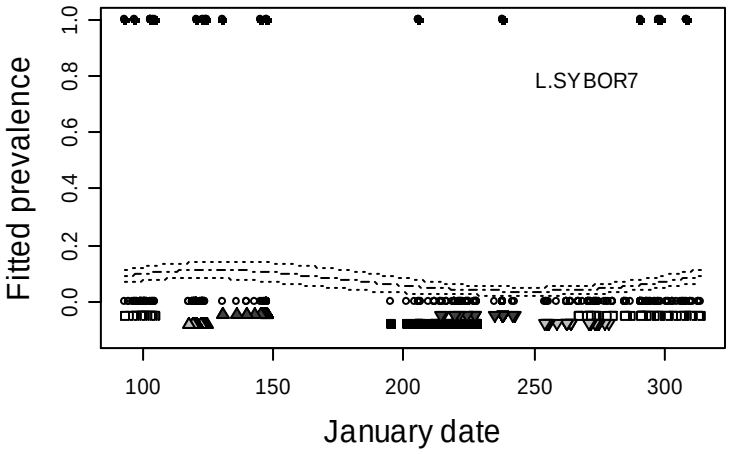


37

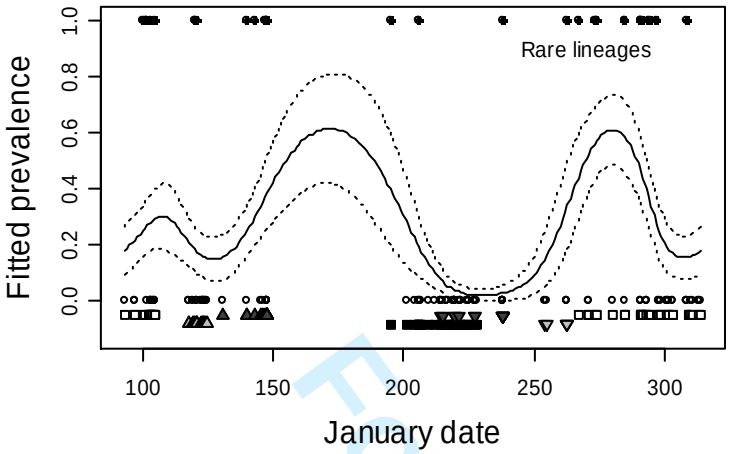
FIGURE 7a-c.





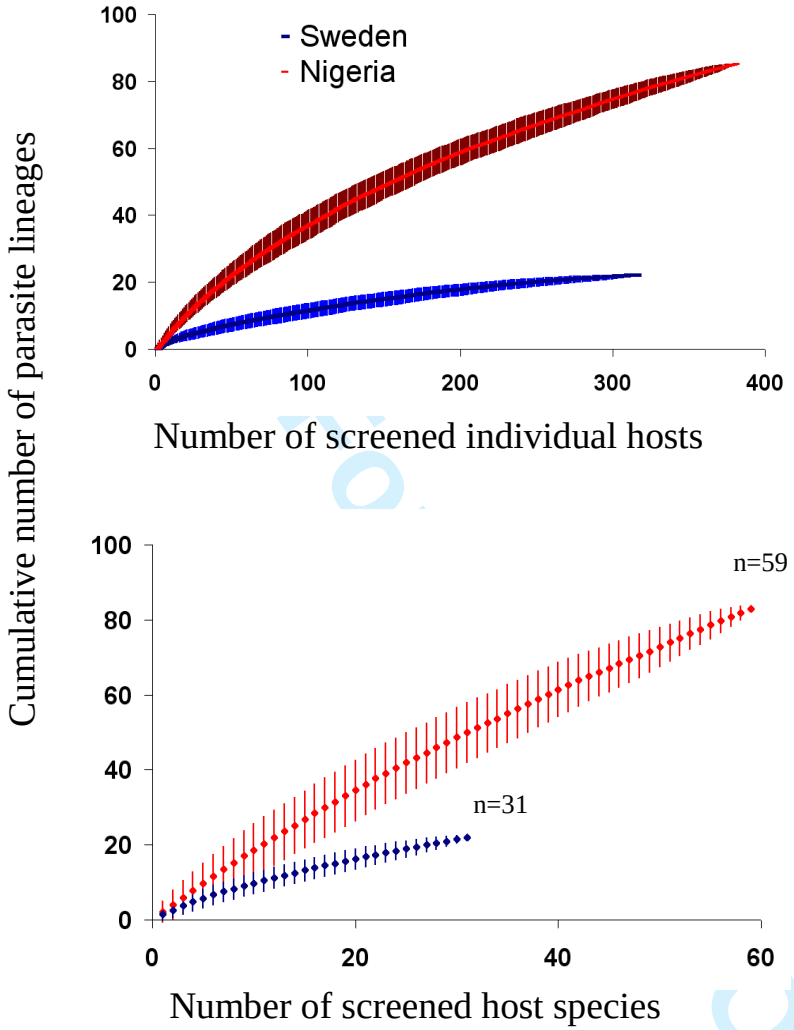


41 FIGURE 8.
42



43

FIGURE 9.


Sweden
Nigeria
Cumulative number of parasite lineages
Number of screened individual hosts
n=59
n=31
Number of screened host species