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ORIGINAL ARTICLE

Temporal patterns of occurrence and transmission of the blood parasite *Haemoproteus payevskyi* in the great reed warbler *Acrocephalus arundinaceus*

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Abstract We studied the prevalence and intensity of the haemosporidian blood parasite *Haemoproteus payevskyi* in great reed warblers at Lake Kvismaren (6 years) and Lake Segersjö (3 years) in Sweden. Based on microscopic inspection of slides from 282 adult birds, 20.6% showed infection of *H. payevskyi* in circulating red blood cells in at least 1 year. For parasite prevalence, there was no difference between years, sex, and age classes. However, parasite intensity was higher in females than in males, and this was most pronounced in 1-year-old birds. Individuals scored to carry parasites in year$_n$ were more likely to show parasite infection year$_{n+1}$ than birds scored to be parasite-free in year$_n$. None of 99 juvenile birds examined at the breeding site in late summer, 4–9 weeks after hatching, showed infection of *H. payevskyi*. Parasite intensity in infected adult birds decreased in the course of the breeding season and no new or relapse infections were observed during this period. Thus, our data imply that in the great reed warbler, a long-distance migrant to tropical Africa, transmission of *H. payevskyi* occurs on wintering sites or at stopover sites during migration.

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Introduction

Passerine birds are often exposed to haemosporidian blood parasites, in particular *Haemoproteus* spp. and *Plasmodium* spp., and the fitness effects of these parasites range from seemingly negligible to severe (Atkinson and van Riper III 1991; Valkiūnas 2005). Many species of passerine birds are exposed to several species and lineages of haemosporidian blood parasites (Bensch et al. 2000; Waldenström et al. 2002; Ricklefs et al. 2004; Pérez-Tris and Bensch 2005). These characteristics of bird host-blood parasite systems provide excellent opportunities for evolutionary and ecological studies, for example, when and where parasite transmission occurs, seasonal patterns of parasite occurrence, and to what extent breeding performance is related to parasitism.

Most ecological studies of blood parasites in birds have been conducted on resident and short-distance migrant species. These studies have usually been focused on the bird hosts, neglecting how different parasite strategies may affect parasite prevalence and intensity. A few studies of long-distance migrating birds at stopover sites show that migrating birds can have parasites in the blood both during spring and fall migration (Peirce and Mead 1978; Valkiūnas 1993; Bennett et al. 1995; Rintamäki et al. 1997; Valkiūnas and Iezhova 2001), and at their tropical wintering and staging sites (Ashford 1971; Davidar and Morton 1993; Bensch et al. 2000; Waldenström et al. 2002, 2004; Hellgren et al., 2007). Hence, because

migrating birds show infections throughout the year, transmission of haemosporidian parasites may not be limited to the breeding season, but may occur also during migration and at (tropical) wintering grounds. For example, in the long-distance migrating purple martin (*Progne subis*), birds carried parasites both at the breeding sites in North America and at the wintering grounds in Brazil (Davidar and Morton 1993), and the same has recently been found in great reed warblers breeding in Europe and wintering in Nigeria (Waldenström et al. 2002). In none of these species is the temporal dynamics of parasitemia known, nor established when transmission occurs. In the great reed warbler, however, circumstantial evidence suggests that some blood parasite species are transmitted in Africa rather than during the breeding period in Europe (Waldenström et al. 2002, 2004; Bensch et al. 2007). Identifying the transmission area of avian blood parasites is important for several reasons. It could for example help us to understand variation in host immunocompetence over the annual cycle (Nelson and Demas 1996). Moreover, it could elucidate to what extent long-distance migrant birds run the risk of contracting blood parasites in (tropical) wintering areas, i.e., to what extent blood parasites can induce a "cost of migration" with negative impact on host population size and viability (Møller and Erritzøe 1998; Waldenström et al. 2002). It could also help us evaluate the risk that migrant birds bring tropical malaria back to their temperate breeding areas where resident birds then may be exposed to these novel parasites, in parallel to the scenario of recent concern for human malaria because global warming is likely to facilitate the spread at more northern latitudes (Rogers and Randolph 2000).

Several studies of birds report on variation in parasite prevalence and intensity, e.g., variation between years (Weatherhead and Bennett 1992; Korpimäki et al. 1995; Bensch and Åkesson 2003; Bensch et al. 2007), temporal variation within years (Weatherhead and Bennett 1991, 1992), as well as age and sex differences (Allander and Bennett 1994; Dawson and Bortolotti 2000; Freeman-Gallant et al. 2001). The causes behind such differences are not well understood and several explanations have been suggested; for example, individual differences in susceptibility (Richner et al. 1995), variation in vector abundance (Allander and Bennett 1994), hormone-induced or activity-related immune system suppression (Folstad and Karter 1992; Wedekind and Folstad 1994; Råberg et al. 1998), strength of sexual selection (Freeman-Gallant et al. 2001), age-dependent differences in acquired immune defence (Gregory et al. 1992; Sol et al. 2003) and frequency-dependent selection between bird hosts' immune system (Mhc) genes and different blood parasite strains (Westerdahl et al. 2005).

In this study, we investigated the occurrence (prevalence and intensity) of the blood parasite *H. payevskyi* in the great reed warbler (*Acrocephalus arundinaceus*), a socially polygynous, long-distance migrant passerine bird breeding in Europe. We analysed the temporal and demographic distribution of *H. payevskyi* in great reed warblers over 6 years to investigate possible differences between sex and age groups as well as between two closely situated (15 km) breeding sites in southern Central Sweden. In particular, we aimed at revealing at what time of the year (and where) the transmission of *H. payevskyi* occurs, an important aspect of host–parasite interactions.

Methods

For the analyses in the present study, we used data collected from great reed warblers at Lake Kvismaren (59°10′N, 15°25′E) in southern Central Sweden in 1991–1993 and 1996–1998, and at lake Segersjö, 15 km east of Lake Kvismaren, in 1996–1998. The Kvismare population has been studied annually since 1985 and the Segersjö population 1994–1998 (Bensch 1996; Hasselquist 1998). Each year at Lake Kvismaren, fieldwork was carried out daily from the arrival of the first great reed warblers in early May to fledging of the last young in the beginning of August. At Segersjö, the population was studied every 1–3 days between May and July. Some individuals, both juveniles and adults, were trapped in the ordinary bird ringing scheme at Lake Kvismaren carried out by Kvismare Bird Observatory each year between July and September (e.g., Hall 1996).

The great reed warbler winters in the sub-Saharan tropical Africa and its breeding range extends from Europe to central Asia (Cramp 1992). The males return to the Swedish breeding grounds in early May and the females about 2 weeks later. The great reed warbler has a facultative polygynous mating system (Dyrcz 1986, 1988); at Lake Kvismaren, about 40% of the territorial males form pair bonds with two to five females, whereas about 20% of the territorial males remain unpaired (Bensch and Hasselquist 1991; Hasselquist 1995, 1998). Each year, a large proportion of the males and all females that had not previously been breeding in our study area (ca. 50% of all breeding females) were captured (using mist nets) soon after arriving to our study area. The remaining males and females were captured later during breeding, in particular during the period when they were feeding nestlings. In spring, previously unringed birds were ringed with a unique combination of three colour rings and one aluminium ring, sexed, and aged into one of two age groups, 1 year old or ≥2 years old (see Bensch et al. 1998). Many individuals, in particular males, were captured at least two

times in a breeding season, which provided us with information about the temporal pattern of blood parasite infection and to what extent transmission may occur during the breeding season. In 1997–1998, effort was also made to capture independent yearling great reed warblers in August–September, i.e., when they were more than 4 weeks old, in order to investigate whether juvenile birds were infected with *Haemoproteus* or *Plasmodium* already before they left the breeding grounds in their first autumn. Each time when an individual was captured we took a blood sample from the brachial or tarsus vein using a cannula. A drop of blood (≈10 μl) was collected in a heparinized microcapillary (20 μl) and the ends of the capillary were sealed with plasticine. Later the same day, a small drop of blood was put on a microscope slide and carefully smeared out. Blood smears were then air-dried for at least 3 min, fixed in pure methanol for 3 min, and later stained in Giemsa staining for 20 min.

In total 455 blood smears were collected from 285 adult great reed warbler individuals (129 males and 156 females). Of these 285 birds, 85 were sampled in more than 1 year (63 were sampled in 2 years, 20 in 3 years, one in 4 years and one in 5 years). We sampled 60 adult birds at least twice in one season. The temporal pattern of blood parasite sampling was rather evenly spread out over the breeding season (mean = 10 June, SD = 16 days, range = 10 May–29 August). We also screened blood smears from 99 juvenile birds sampled when 4–9 weeks old.

All blood smears were intensively searched for *Haemoproteus* and *Plasmodium* parasites by Ö. Östman. In total, 69 individuals showed infections with *Haemoproteus* and *Plasmodium*. It is often difficult to identify species of *Haemoproteus* and *Plasmodium* from blood smears, in particular as in our case when blood had been smeared and stained some hours after being collected. We therefore checked parasite lineage/species by PCR amplification and sequencing of the cytochrome *b* gene of the parasite mitochondria in 67 of the 69 individuals (97%) found to carry blood parasites when examining blood smears (for details of molecular methods, see Bensch et al. 2000; Waldenström et al. 2002, 2004). An overwhelming majority of the blood smear positive great reed warblers in our study population had identical DNA sequences at the cytochrome *b* gene (64 out of 67 had lineage GRW1; GenBank accession no. AF254964), and this blood parasite was then identified as *H. payevskyi* by microscopic investigation of blood smears (carried out by G. Valkiūnas). Only two individuals showed infection by *P. ashfordi* (lineage GRW2; GenBank accession no. AF254962; Valkiūnas et al. 2007); note, however, that this screening is based on microscopic investigation of blood smears—our PCR-based studies show that *Plasmodium* infections of low intensity are much more common in this population (see also Waldenström et al. 2004; Bensch et al. 2007). The remaining bird was infected by another, so far unidentified *Plasmodium* species (lineage GRW6; GenBank accession no. DQ368381; Bensch et al. 2007). In the present study, we have concentrated on investigating patterns of occurrence and transmission of *H. payevskyi*. In the analyses, we have excluded the birds infected with other haemosporidian blood parasites ($n = 3$) and the infected individuals where DNA was not available for haemosporidian species identification ($n = 2$). Thus, the remaining data set used for the analyses of occurrence of *H. payevskyi* infections consisted of 388 samples (177 males and 211 females, one sample per bird and year) collected from 282 different individuals of adult great reed warblers (of which 61 were sampled in 2 years, 19 in 3 years, 1 in 4 years, and 1 in 5 years).

Parasite intensity was estimated by using a light microscope with 1,000× magnification (oil immersion) counting the number of blood parasites in 7,500–8,000 red blood cells in an area of the blood smear with homogenous dispersion of cells. The number of red blood cells was estimated as the mean of the first and last field multiplied by the number of fields. Blood smears were scored without knowing the identity of the sampled bird. From 34 infected birds, blood parasites were counted twice (from different areas of the same slide) to estimate repeatability (Lessells and Boag 1987). The repeatability was high ($R = 0.95$, $F_{1,32} = 42.0$, $P < 0.001$). Parasite intensity was log-transformed to normalise the skewed distribution. For the statistical analyses we used the PC software SYSTAT 10.0 (SPSS Inc. 2000) and methods described in Sokal and Rohlf (1981). When analysing parasite prevalence in relation to time of the breeding season and between years, we conducted logistic regressions. In analyses of parasite intensity, we used generalised linear model analyses and performed stepwise backward elimination of non-significant factors and interactions ($P > 0.05$). Percentage values of parasite intensity were arcsin square root transformed before being included in the parametric tests. All statistical tests are two-tailed.

Results

Based on investigations of blood smears, 58 individuals (30 males and 28 females), i.e., 20.6% of all investigated adults ($n = 282$), showed infection with *H. payevskyi* in at least one breeding season. The overall prevalence was 17.5% (SE = 1.7).

Parasite intensity, i.e., the proportion of host red blood cells infected with *H. payevskyi*, varied from 0.011 to 8.4% (mean = 0.58%, SE = 0.15, median = 0.18%, $n = 64$; six

birds included from 2 years). The annual mean parasite intensity was 0.61% (SE = 0.17). The two birds carrying *P. ashfordi* (lineage GRW2) had parasite intensities of 0.022 and 1.19%.

Patterns within a breeding season

Parasite prevalence

The great reed warblers had gametocytes of *H. payevskyi* in the red blood cells at the time of their arrival to the breeding sites in Sweden. There was no significant difference in parasite prevalence depending on how long time after arrival birds were sampled (logistic regression: *t*-ratio = 1.02, $P = 0.31$, $N = 254$ birds with known arrival dates and each bird included only once). When we did a similar analysis based on date of sampling, instead of the time interval between arrival and sampling, again there was no significant change in parasite prevalence over the season (logistic regression: *t*-ratio = 0.02, $P = 0.99$, $n = 254$). The same was true for the relationship between arrival date and parasite prevalence (logistic regression: *t*-ratio = 0.02, $P = 0.99$, $n = 254$). None of the birds scored to be parasite free at the first sampling date were found to carry *H. payevskyi* gametocytes at a later date within the same year (48 cases involving 44 different birds).

We also investigated blood smears of 99 juvenile birds sampled when they were 4–9 weeks old. These juveniles originated from 52 different broods and were previously ringed as nestlings at Lake Kvismaren. In none of these juveniles did we find any haemosporidian gametocytes in the blood. The 52 broods involved 64 different parents of which ten had shown infections by *H. payevskyi* the actual year.

Parasite intensity

To investigate which factors affected the intensity of infection of *H. payevskyi* ($n = 64$; three males and three females included more than 1 year), we conducted generalised linear model analyses with the independent factors sex, age, sampling date, study site, and their interactions (Table 1). All non-significant interactions were excluded, as well as the two non-significant factors sampling date ($P = 0.75$) and study site ($P = 0.90$). The final model included the significant factors sex ($P < 0.001$), age ($P < 0.001$), and the interaction between sex × age ($P = 0.0052$). We therefore reanalysed the data on the relationship between age and parasite intensity for males and females separately. In males ($n = 33$), parasite intensity only tended to be higher in 1-year-old as compared with ≥2-year-old birds ($P = 0.089$; Fig. 1a). In females ($n = 31$), parasite intensity was clearly higher in 1-year-old birds ($P = 0.0002$; Fig. 1b). Arrival date also did not contribute significantly to the male or the female model ($P > 0.45$). The parasite intensity of females (mean = 0.86%, SE = 0.29) was higher than for males (mean = 0.32%, SE = 0.08; $t_{62} = 2.06$, $P = 0.046$; Fig. 1).

In birds captured two times the same year, 13 out of 16 birds (81%) had higher parasite intensity in their first sample (mean = 0.72%) as compared with their second sample (mean = 0.12%) of the season (Sign test, $P = 0.021$; average number of days between first and second capture was 17.0 ± 3.0 days, median date for first capture = 27 May and for second capture = 11 June).

Table 1 Results of ANCOVAs relating parasite intensity of *Haemoproteus payevskyi* to sex, age, and days to sampling (i.e., number of days between arrival and sampling), as well as possible interactions between the factors (total model: $F_{5,59} = 7.4$, $P < 0.001$)

	All ($n = 65$)		Males ($n = 32$)		Females ($n = 33$)	
	F	*P*	*F*	*P*	*F*	*P*
Sex	14.7	<0.001				
Age	3.1	0.084	0.4	0.55	10.0	0.004
Days after arrival (DAA)	3.4	0.072	6.6	0.016	1.2	0.28
DAA × age	4.8	0.033	4.4	0.045	1.93	0.18
Sex × age	13.3	<0.001				

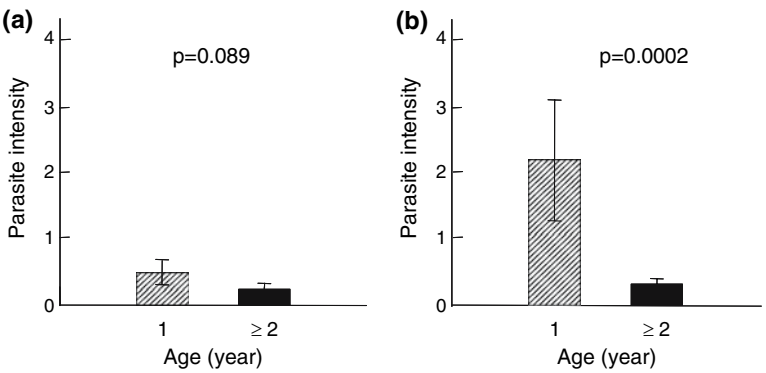

Fig. 1 Parasite intensity of great reed warblers infected with *Haemoproteus payevskyi* in relation to host age (1-year-old or ≥2-year-old) for **a** females (1-year-old $n = 9$; ≥2-year-old $n = 22$) and **b** males (1-year-old $n = 11$; ≥2-year-old $n = 22$). Mean values ± SE

Moreover, out of 16 birds scored as parasitized in the first sample, three did not show any *H. payevskyi* gametocytes when sampled the second time of the season. These three birds had among the lowest intensities in their first sample (0.016–0.069%) and relatively long period of time elapsed between the first and second sampling (11–31 days). The bird with the highest infection intensity in our study (8.4% of infected red blood cells) survived and returned to breed the following year.

Prevalence patterns between breeding seasons

In a logistic regression including each individual once every year ($n = 388$), we found no difference in parasite prevalence between years ($P = 0.18$), sex ($P = 0.23$), or age classes ($P = 0.67$), but there was a difference between study sites ($P = 0.033$). Including each individual only once (their first breeding season) in the analysis resulted in very similar results. The prevalence in Segersjö was higher (28.6%, $n = 49$) than in Kvismaren (14.7%, $n = 339$).

Among birds captured in 2 years, there was a higher probability for a bird to retain its prevalence status between years than changing prevalence status (going from infected to non-infected and vice versa; $\chi^2_{(1)} = 39.2$, $P < 0.0001$, $n = 101$, 21 birds included two times but including each bird only once give similar results). Nine parasitized and 77 unparasitized birds retained their infection status between years, whereas 5 birds gained and 10 lost infections.

Discussion

In this study, we used molecular methods to securely identify that all parasitized great reed warblers included in our study were infected by *H. payevskyi*. Combined with microscopic analyses of smears, this allowed us to obtain reliable data on prevalence (20.6%) and intensity (mean = 0.58%) of *H. payevskyi* in this host species. Moreover, we were also able to infer the seasonal pattern of transmission of this blood parasite.

Between-year patterns

There was no significant difference in parasite prevalence between years. In the few studies of parasite prevalence in birds covering several years, annual differences have often been found for at least one sex or age group (Weatherhead and Bennett 1991, 1992; Allander and Bennett 1994; Korpimäki et al. 1995). The rather high and consistent prevalence level and moderately high parasite intensity of *H. payevskyi* in great reed warblers may suggest that the parasite and host have coevolved for a long period of time and that the parasite at present is rather benign to its host. For more virulent parasites, we predict that higher parasite-induced mortality would have caused fluctuations in parasite prevalence between years and generally lower parasite intensity in infected birds (that survived to be examined).

We found evidence that individual birds retained infection status between years, i.e., a bird that was parasitized in $year_n$ were also parasitized in $year_{n+1}$ and vice versa. This may not solely be an effect of *H. payevskyi* infections being chronic, because more than 50% of the infected birds in $year_n$ appeared to be parasite free in $year_{n+1}$ (see also Weatherhead and Bennett 1991, 1992; Dale et al. 1996). These data suggest that in great reed warblers there are individual differences in susceptibility to *H. payevskyi* infections. Such individual differences could be due to differential exposure to vectors carrying the parasite or caused by differences in genetically based or condition-dependent resistance of the host (Richner et al. 1995; Westneat and Birkhead 1998). So far, we have not been able to link prevalence of *H. payevskyi* to certain genotypes at the major histocompatibility complex (Mhc; i.e., genes encoding antigen-recognizing proteins in the vertebrate immune system) in adult great reed warblers (Westerdahl et al. 2005), which may argue against a genetically based resistance.

Within-season patterns

For both males and females, parasite intensity was highest shortly after they arrived in spring, before the main mating period, and it then decreased during the course of the breeding season. In some other investigated passerine birds, parasite intensity has been stable over the season (Weatherhead and Bennett 1991; Dale et al. 1996) or peaked during the early breeding or nestling feeding periods (Allander and Sundberg 1997; Oppliger et al. 1997). In the brown-headed cowbird, parasite intensity decreased during the breeding season while parasite prevalence increased (Weatherhead and Bennett 1992). The observed decline in parasite intensity over the breeding season in great reed warblers is an unexpected result, because it is generally thought that high workload and stress during territory settlement, mate attraction, and egg laying, in combination with hormonal changes associated with breeding, would lead to suppressed immune function in hosts and hence facilitate relapses of infections (Atkinson and van Riper III 1991).

We suggest two hypotheses that might explain the decrease in parasite intensity over the season in great reed warblers: (1) adaptive parasite transmission, and (2) suppressed host immune function. The first hypothesis assumes that haemosporidian parasites are active mainly during favourable conditions for transmission, i.e., at high vector and host abundance just before or during the spring

migration. The second hypothesis would suggest that great reed warblers have a more suppressed immune defence during migration and when they arrive at the breeding grounds than later during the breeding period. These two explanations may not be mutually exclusive, but may interact to shape the pattern of parasite infection that we observe when investigating blood smears.

When a host–parasite coevolution system is in equilibrium, the parasites should only produce gametocytes when transmission is possible (the adaptive transmission hypothesis). Two factors have an important impact on the probability of transmission; the availability of appropriate vectors (most probably *Ceratopogonidae* for *Haemoproteus*) and the abundance of hosts (great reed warblers and possibly closely related *Acrocephalus* species that may act as hosts; Bensch et al. 2000; Valkiūnas 2005). If the conditions for transmission of *Haemoproteus* are very low at the breeding grounds, for example due to absence or low abundance of vectors and/or hosts, parasite infections are likely to be latent. Then, the gametocytes in the blood would just be those remaining from an earlier outbreak, and the decrease in parasite intensity over the season a consequence of the normal replacement of red blood cells in the host, which takes 35–45 days in passerines (Sturkie and Benzo 1986).

On the other hand, *Haemoproteus* may produce gametocyte whenever the host's immune system is weakened independent of the probability of transmission (the suppressed host resistance hypothesis). The decrease in parasite intensity over the breeding season could then be a consequence of seasonally enhanced immunocompetence in great reed warblers. It is known that stress (e.g., strenuous exercise) and steroid hormones can suppress immune defences (Folstad and Karter 1992; Wedekind and Folstad 1994; Råberg et al. 1998). If these factors affect immune function in great reed warblers, it suggests that migration and prebreeding behaviours are more stressful than breeding behaviours in this long-distance migrant. Indeed, migratory restlessness has been shown to induce relapse infections of the *Borrelia burgdorferi* bacterium in redwings *Turdus iliacus* (Gylfe et al. 2000). However, in a wind-tunnel experiment on red knots *Calidris canutus*, flying 1,500 km in a week did not suppress the birds' immune responses (Hasselquist et al., 2007). Moreover, in another long-distance migrant, the pied flycatcher, there was no change in intensity of *Haemoproteus* infections over the breeding season (Dale et al. 1996). Hence, taken together these data seem to favour the adaptive transmission hypothesis.

Sex, age, and site patterns

We found differences in parasite intensity, but not parasite prevalence, between sex and age groups of great reed warblers. It is interesting to note that in a study on feral pigeons, intensity but not prevalence of *H. columbae* differed among age groups (Sol et al. 2000, 2003).

One-year-old great reed warblers had higher *H. payevskyi* infection intensities than older birds, a relationship that was more pronounced in females. This could reflect that the immune systems of 1-year-old birds could not cope as efficiently with *H. payevskyi* infections as that of adult birds, or that 1-year-old birds were more susceptible to the demands of spring migration with adverse effects on their general condition.

Female great reed warblers had higher parasite intensity than males, despite that the study populations of great reed warblers show a rather high level of social polygyny (35–40% of territorial males form pairbonds with two to five females; Bensch 1996; Hasselquist 1998), and males thus are subjected to a considerable sexual selection pressure (Hasselquist et al. 1996; Hasselquist 1998). This result does not agree with the general finding in vertebrates (including birds) that females have higher immunocompetence and are less parasitized than males (Zuk 1990; Folstad and Karter 1992; Olsen and Kovacs 1996). Our finding in great reed warblers is even more surprising given that it has been hypothesized and empirical evidence presented for a male-biased parasite exposure in species with higher levels of sexual selection (Zuk 1990; Zuk and McKean 1996; Freeman-Gallant et al. 2001), and that male great reed warblers invest substantial time and energy into singing their mate attraction song from spring arrival at the breeding site onwards for 1–2 months (Hasselquist et al. 1993; D. Hasselquist and S. Bensch, unpublished). A possible explanation is that in this socially polygynous species females often care for egg and offspring alone without male help (Bensch and Hasselquist 1994; Sejberg et al. 2000). However, this explanation is unlikely because females had higher parasite intensity already at arrival to the breeding site, i.e., before the main period of parental care. Another important difference between the sexes is that the females arrive on average 10–14 days later to the breeding grounds and thus probably stay longer in Africa than the males. Interestingly, 1-year-old birds also had higher infection intensities and they also arrive ca. 2 weeks later to the breeding grounds, suggesting that they stay longer in Africa than the adult males. Still, we cannot explain why a longer stay in Africa in spring would affect parasite intensity, but not parasite prevalence.

Transmission

None of the 99 juvenile birds investigated at the breeding grounds when 4–9 weeks old showed any sign of *H. payevskyi* infection. As the incubation time for *Haemoproteus* is ca 2 weeks (Desser and Bennett 1993), there should have

been plenty of time for a juvenile bird to become infected and for the parasite to develop gametocytes in the blood. In brown-headed cowbirds (*Molothrus ater*), less than 4-week-old juveniles showed infection of Haematozoa, including *Haemoproteus*, with parasite intensities comparable to that of adult conspecifics (Weatherhead and Bennett 1992). Pied flycatcher (*Ficedula hypoleuca*) nestlings at 13 days of age showed high parasite intensity of *Haemoproteus* and *Trypanosoma* and medium levels of parasite prevalence compared to adults (Merino and Potti 1995). In purple martins, however, none of 35 nestlings investigated when 25–28 days old showed any blood parasite infection (Davidar and Morton 1993). The lack of haemosporidian infections in juvenile great reed warblers during the post fledging period strongly implies that they had not yet been exposed to the *H. payevskyi* blood parasite. The alternative interpretation, that they had been exposed to these parasites, but that the infection remained latent is highly unlikely because a host individual's first exposure to a blood parasite usually results in moderate to high intensities (Valkiūnas 2005). This fact together with the high prevalence (17% of breeding birds are infected) and the high number ($n = 99$) of investigated juvenile birds makes it exceedingly unlikely that we should have failed to detect infections if there had been any.

Most great reed warblers arrive at their Swedish breeding sites in May. Even if transmission occurred among newly arrived birds, gametocytes would not be visible in the blood until about 2 weeks later. Hence, because a substantial proportion of the great reed warblers carried *H. payevskyi* gametocytes shortly after arriving at their breeding grounds, they must either have become infected earlier, during winter or early spring, or during the preceding breeding season. If transmission takes place during the breeding season, juvenile birds must have been infected shortly after hatching the previous season because we observed *H. payevskyi* in 1-year-old birds caught within 2 weeks after arrival the subsequent spring (16 out of 91 birds were infected). If, however, transmission occurs outside the breeding season, we would expect to see only relapses and no new infections in adult birds at the breeding grounds. Our data support the latter hypothesis because in 30 individuals scored parasite free when captured shortly after arrival to the breeding site in spring, none were infected when recaptured 3–9 weeks later when new infections contracted at the breeding site should have been detectable (exact probability test: $P = 0.004$).

Ashford (1971) found high prevalence of Haematozoa, including *Haemoproteus*, in yellow wagtails (*Motacilla alba*) and whitethroats (*Sylvia communis*) investigated in Nigeria just prior to their northward migration in spring, a period when migrating birds are under considerable physiological stress. Hence it is possible that transmission occurs at the wintering grounds. This would be even more likely if there is a high density of great reed warblers and suitable vectors at wintering grounds facilitating the transmission of haemosporidia. Great reed warblers show high site fidelity and can reach quite high local densities at suitable wintering sites in tropical Africa (De Roo and DeHeegher 1969; Hedenström et al. 1993). Moreover, the *H. payevskyi* cytochrome *b* sequence clusters with closely related *Haemoproteus* parasites that have been found in West African resident bird species (Waldenström et al. 2002; Bensch et al. 2007) which provide further support for transmission of these parasites during the non-breeding season in Africa. Also data from other *Acrocephalus* species suggest that the transmission of *H. payevskyi* takes place in the African winter quarters or on stopover sites during migration (Valkiūnas 2005).

To conclude, the available data imply that transmission of *H. payevskyi* occurs at wintering grounds in Africa or at stopover sites during migration and that no transmission takes place during summer in Europe. This finding has important ecological implications. First, it shows that certain blood parasites can have their transmission areas restricted to the tropical winter quarters of their bird host, and hence have the potential to induce a "cost of migration" at present or in the past during the coevolution of the parasite and its host. Second, it highlights the potential for tropically transmitted blood parasites to hitchhike with long-distance migrants to Europe in summer and there, e.g., due to global warming, be able to infect resident birds previously not exposed to the parasite. In novel bird hosts, seemingly benign blood parasites may have severe negative consequences on population density (Atkinson and van Riper III 1991).

Zusammenfassung

Wir untersuchten die Prävalenz und die Befallsintensität von Drosselrohrsängern mit dem Blutparasiten *Haemoproteus payevskyi* (Haemosporida) am Kvismaren-See (über einen Zeitraum von 6 Jahren) und am Segersjö-See (3 Jahre lang) in Schweden. Auf Grundlage einer mikroskopischen Untersuchung von Blutausstrichen von 282 Altvögeln zeigten 20,6% mindestens in einem Jahr eine Infektion der Erythrozyten mit *H. payevskyi*. Bezüglich der Prävalenzwerte ergaben sich zwischen den Jahren, Geschlechtern und Altersklassen keine Unterschiede. Allerdings lag die Befallsintensität bei Weibchen höher als bei Männchen, was bei einjährigen Vögeln am deutlichsten ausfiel. Individuen, bei denen im Jahr n der Parasitennachweis positiv ausgefallen war, zeigten mit höherer Wahrscheinlichkeit auch im Jahr n+1 eine Parasiten-Infektion, als Individuen, in denen im Jahr n keine Parasiten

gefunden worden waren. Kein einziger der 99 Jungvögel, die im Brutgebiet im Spätsommer, 4 bis 9 Wochen nach dem Schlupf untersucht wurden, war mit *H. payevskyi* infiziert. Die Befallsintensität ging bei den infizierten Altvögeln mit Voranschreiten der Brutsaison zurück und während dieser Zeit wurden auch keine Neuinfektionen oder Rezidive beobachtet. Folglich legen unsere Ergebnisse nahe, dass die Übertragung von *H. payevskyi* auf Drosselrohrsänger, Langstreckenzieher ins tropische Afrika, im Winterquartier oder in Rastgebieten auf dem Zug erfolgt.

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