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Cross-species infection of blood parasites between resident and migratory songbirds in Africa

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Abstract

We studied the phylogeny of avian haemosporidian parasites, *Haemoproteus* and *Plasmodium*, in a number of African resident and European migratory songbird species sampled during spring and autumn in northern Nigeria. The phylogeny of the parasites was constructed through sequencing part of their mitochondrial cytochrome *b* gene. We found eight parasite lineages, five *Haemoproteus* and three *Plasmodium*, infecting multiple host species. Thus, 44% of the 18 haemospiridian lineages found in this study were detected in more than one host species, indicating that host sharing is a more common feature than previously thought. Furthermore, one of the *Plasmodium* lineages infected species from different host families, Sylviidae and Ploceidae, expressing exceptionally large host range. We mapped transmission events, e.g. the occurrence of the parasite lineages in resident bird species in Europe or Africa, onto a phylogenetic tree. This yielded three clades, two *Plasmodium* and one *Haemoproteus*, in which transmission seems to occur solely in Africa. One *Plasmodium* clade showed European transmission, whereas the remaining two *Haemoproteus* clades contained mixes of lineages of African, European or unknown transmission. The mix of areas of transmission in several branches of the phylogenetic tree suggests that transmission of haemosporidian parasites to songbirds has arisen repeatedly in Africa and Europe. Blood parasites could be viewed as a cost of migration, as migratory species in several cases were infected with parasite lineages from African resident species. This cost of migration could have considerable impact on the evolution of migration and patterns of winter distribution in migrating birds.

Keywords: cost of migration, cytochrome *b*, *Haemoproteus*, host shift, mitochondrial DNA, parasite transmission, *Plasmodium*

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Introduction

The vector-borne avian blood parasites of the genera *Haemoproteus* and *Plasmodium* have almost worldwide distributions. Based on morphological classification, these two parasite genera include a high diversity of species (Atkinson & van Riper 1991). The prevailing hypothesis proposes that the high species diversity is a consequence of very high host specificity, particularly in *Haemoproteus* (Atkinson & van Riper 1991). Recently, using a polymerase chain reaction (PCR)-based protocol to study mitochondrial DNA (mtDNA) variation at the cytochrome *b* gene of avian haemosporidian parasites, Bensch *et al*. (2000) found one case of a single parasite lineage infecting two closely related bird species. In addition, the rather low similarity of the phylogenetic trees of the parasites and their bird hosts suggested that host shifts had occurred repeatedly in the evolution of this parasite and host system. However, it is not known how common cross-species transmission is. Here we examine the possibility of cross-species transmission between relatively more distantly related bird host species coexisting in the same habitat. If cross-species transmission is a regular feature of this host–parasite system, we would expect to find more cases in which one parasite lineage infects multiple host species.

The ecological consequences of parasite infections on hosts have been intensively studied in breeding birds (e.g.

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Dale *et al*. 1996; Nordling *et al*. 1998; Merilä & Andersson 1999; reviews: McCurdy *et al*. 1998; Norris & Evans 2000). However, the potential costs that parasites may inflict as a consequence of migratory habits have been less studied (Clarabuch & González-Solís 1998). A specific cost could involve resource allocation from fat deposition to behaviours that combat the parasite infection (Valkiunas 1993a). More generally, however, many migratory birds move between vastly separated areas, and thereby encounter two different faunas of parasites and pathogens compared with resident species. In particular long-distance migrants that winter in tropical areas are likely to encounter diverse parasite faunas (Møller & Erritzøe 1998). In the Palaeartic–African bird migration system, resident birds in Africa may act as reservoirs for tropical avian blood parasites (Valkiunas 1993b), increasing the risk for Palaearctic migrants to become infected immediately when reaching their African winter quarters. Another factor potentially enhancing the parasites' adverse effects on long-distance migrant birds is their exposure to strenuous physical activity during their migratory journey. This may result in a down-regulation of the immune system, either to allocate more energy to migration activities (Sheldon & Verhulst 1996) or to avoid stress-induced immunopathology (Råberg *et al*. 1998). Hence, if cross-species transmission occurs at wintering sites, this would be a new and potentially high cost of migration.

We have had the opportunity to study cross-species transmission of avian haemosporidians at a site in tropical Africa (north-eastern Nigeria), where the bird community consists of both European migrants and African resident bird species. European long-distance migrants can potentially become infected by *Haemoproteus* and *Plasmodium* blood parasites both in their European breeding areas and in their African winter quarters (Valkiunas 1993b). This parasite–host system is therefore suitable as a model when exploring the role of parasitism for the cost of migration.

For *Haemoproteus* and *Plasmodium* parasites to have an impact as a cost of migration the following criteria must be fulfilled: (i) the migrants are exposed to the parasites at the winter quarters; (ii) the parasites can infect multiple host species from both resident and migrant bird species, and (iii) parasitaemia is associated with fitness costs in the host. Costs of being infected with avian haemosporidian parasites can be quite severe. In particular *Plasmodium* can have a strong negative impact on its host (Richner *et al*. 1995), especially when a bird species is exposed to these parasites for the first time (van Riper *et al*. 1986). The adverse effects of a *Haemoproteus* infection are less pronounced, but several studies clearly show that costs may occur (Atkinson *et al*. 1988; Nordling *et al*. 1998; Merilä & Andersson 1999). In this study, we explore the host specificity of *Haemoproteus* and *Plasmodium* in a phylogenetic context using molecular methods, and discuss the temporal timing of transmission, as well as the differences in parasite prevalence between resident and migratory bird species. In particular, we investigate the extent to which cross-species transmission of identical cytochrome *b* lineages of avian haemosporidian parasites occurs, and to what degree there is sharing of parasites between migrant and resident birds that coexist in tropical Africa during the nonbreeding season.

Materials and methods

Study area and sampling procedure

The field study was carried out in northeastern Nigeria, in the area around the town of Malamfatori (13°33′N, 13°23′ E), close to Lake Chad. This is within the Sahel zone, and the habitat consists mainly of dry savannah with few scattered *Acacia tortilis* and *A. senegal* trees. The vegetation undergoes large seasonal changes due to variation in precipitation. The short rainy season in August–September is followed by many months without rain. In spring, the weather is very hot and dry, with nearly all vegetation being withered, whereas the autumn is less hot and more humid, with lush vegetation and water in the rivers. Birds were trapped at two localities during spring (February–May) and autumn (August–November) 2000; one dry inland locality with large sand dunes dominated by dense stands of the saltbush *Salvadora persica*, and one locality at Lake Chad shore where large areas of introduced *Mimosa* spp. trees have been drowned by the rising lake level, giving a mixture of water, bushes and farmland.

The study area is mainly a stopover site for migratory European birds: in spring before, and in autumn after crossing the Saharan desert. However some species, e.g. sub-Alpine warbler *Sylvia cantillans*, lesser whitethroat *S. curruca* and common whitethroat *S. communis*, remain in the area throughout the winter (Fry *et al*. 1970). The resident bird fauna is relatively poor in number of species compared with more tropical areas, with a few species dominating in numbers.

Birds were trapped using mistnets during the early morning, and sometimes also during the evenings. Blood samples were taken from a subset of the trapped bird species, both Afrotropical resident species (n = 104 individuals of seven species; hereafter termed residents) and European migrant species (n = 246 individuals of nine species, hereafter termed migrants). The sampled species included came from five different genera: *Acrocephalus*, *Hippolais*, *Phylloscopus*, *Cisticola* and *Prinia*. The samples, ≈ 20 μL blood taken from the brachial vein, was stored in SET-buffer and were held at ambient temperatures in the field, and later in the laboratory at –80 °C. In this study, most migrant species could be aged according to differences in feather shape and wear (Svensson 1992), enabling separation of

juvenile and adult birds in autumn, and yearling and adult birds in spring. In resident species, age-specific plumage characters are less well known. The seasonality of breeding in resident species has been poorly studied, which makes classifications used in European species difficult to apply. However, the appearance of brood patch in breeding birds, presence of juvenile down and the state of scull ossification in juvenile birds (Svensson 1992) were used to age resident birds after the rain season in autumn. No criteria were available in spring. We therefore only used the autumn data set to compare parasite prevalence among different age groups.

Genetic analyses

Purified DNA was obtained following digestion by proteinase K, and extraction with phenol and chloroform (Sambrook *et al*. 1989). Infection of *Haemoproteus* or *Plasmodium* was detected with PCR amplification of a part of the cytochrome *b* gene of the parasites' mitochondria, using the primers HAEMF and HAEMR2 (Bensch *et al*. 2000). We used a PCR protocol developed by Bensch *et al*. (2000), but lowered the annealing temperature to 50 °C instead of 55 °C. The PCR products were separated on 2% agarose gels, and a fragment of 525 bp was found in individuals infected with haemosporidian parasites (i.e. birds having gametocytes or sporozoites in their blood stream). In a few cases, when only faint bands appeared in the gel, we optimized the PCR by varying the annealing temperature (between 48 °C and 58 °C) and concentration of $MgCl_2$ (1.5–4.0 mM).

All samples with positive amplification were prepared for sequencing by ethanol precipitation. The fragments were sequenced directly from the 5'-end with the primer HAEMF using dye terminator cycle sequencing (big dye) and loaded on an ABI Prism™ 310 (Perkin–Elmer).

Sequences were edited and aligned using the program BIOEDIT (Hall 1999). A sequence divergence of at least one base was used to define lineages. Phylogenetic relationships between obtained and published (Escalante *et al*. 1998; Bensch *et al*. 2000) *Haemoproteus* and *Plasmodium* lineages, together with previously unpublished sequences from Europe and Africa (Bensch unpublished), were estimated using the program MEGA (Kumar *et al*. 1993) and the neighbour-joining method. We used a Kimura 2-parameter distance for the DNA sequence data under a gamma distribution ($\alpha = 0.24$). Primate and human malaria parasites were used as outgroups; *Plasmodium falciparum* (GenBank Accession no. AF069609), *P. reichenowi* (AF069610), *P. ovale* (AF06925) and *P. malarae* (AF069624). Bootstrap values were counted as percentage of replicates over 500 replications. Names of lineages and first detected host species are given in Appendix. The sequence of *Haemoproteus columbae* was obtained from a *Columba livia* sampled in Botswana, which showed high levels of parasite infection on blood smears (EZ Mushi personal communication).

The sequences have been deposited in the GenBank International Nucleotide Sequence Database with Accession nos AF495547–AF495580.

Results

We encountered 105 individuals positive for either *Haemoproteus* or *Plasmodium* out of 350 tested individuals (Table 1). These positive samples represented 18 different mitochondrial lineages obtained from nine species of birds.

Differences in prevalence between taxa

The prevalence of blood parasites (% infected birds) varied widely between species from 0 to 100% (Table 1). In the two genera *Cisticola* ($n = 28$) and *Prinia* ($n = 10$), which include three resident species (Table 1), no parasites were detected. In contrast, in the genera *Acrocephalus* and *Hippolais*, in which some species are resident and some migratory (Table 2), we found moderate or high levels of prevalence in most species (Table 1). We captured only a few individuals of three species of *Phylloscopus* warblers, which are all migratory, and encountered parasites in one but not in the other two (Table 1).

Differences in prevalence between age classes in autumn

Among *Acrocephalus*, for which we have the largest data set, 40.0% of resident juveniles ($n = 10$) and 62.5% of resident adults ($n = 8$; $\chi^2_1 = 0.9$; n.s.) were infected during autumn (two species combined). In migratory *Acrocephalus*, 8.5% of the juveniles ($n = 47$) and 54.1% of the adults ($n = 37$; $\chi^2_1 = 22.0$; $P < 0.001$) were infected during autumn (three species combined). Among *Acrocephalus*, there was no difference in parasite prevalence between resident and migrant adult birds ($n = 45$; $\chi^2_1 = 0.19$; n.s.), but there was a significant difference between migratory and resident juveniles ($n = 57$; $\chi^2_1 = 6.57$; $P = 0.01$).

Differences between spring and autumn

To investigate whether bird species showed a temporal variation in parasite prevalence, we plotted prevalence in autumn against prevalence in spring, including the eight species of which we had sampled at least four individuals in both seasons (Fig. 1). There was a significant correlation ($r_s = 0.76$, $P < 0.05$) between prevalence in autumn and prevalence in spring, suggesting time-independent

Table 1 Number of examined birds, number of individuals parasitized by *Haemoproteus* or *Plasmodium* and prevalence (%), for bird species trapped in Nigeria during spring and autumn 2000. The data for the Olivaceous Warbler is divided into its two subspecies. Also shown are the identified haemosporidian parasite lineages found in each bird species

	Spring				Autumn			
Species	Exam.	Para.	%	Lineage	Exam.	Para.	%	Lineage
Acrocephalus								
Great reed warbler	44	2	4.5	GRW1	8	1	12.5	GRW1
Greater swamp warbler	1	1	100	LSW1	—	—	—	
Lesser swamp warbler	11	3	27.3	LSW1	13	9	69.2	GRW2, GRW4, LSW1, LSW2
African reed warbler	6	4	66.7	MW1, ARW1	14	4	28.6	GRW4, MW1, SW1
Eurasian reed warbler	30	5	16.7	MW1, SW1, ARW1	24	3	12.5	SW1, SGS1, RW2
Sedge warbler	44	13	29.5	GRW4, SW1, SW2, SW3, SW5	54	20	37.0	SW1, SW2, SW3, SW5, SGS1
Marsh warbler	—	—	—		2	0	—	
Hippolais								
Icterine warbler	1	1	100	HIICT2	20	9	45.0	HIICT1
Olivaceous warbler (*laeneni*)	10	4	40.0	HIP2	11	2	18.2	GRW4, HIP2
Olivaceous warbler (*opaca*)	8	2	25.0	HIP2, HIP4	3	0	—	
Cisticola								
Winding cisticola	11	0	—		9	0	—	
Zitting cisticola	4	0	—		4	0	—	
Prinia								
River prinia	5	0	—		5	0	—	
Phylloscopus								
Willow warbler	—	—	—		5	0	—	
Bonelli's warbler	—	—	—		1	0	—	
Wood warbler	—	—	—		2	2	100	PHSIB1
	175	55			175	50		

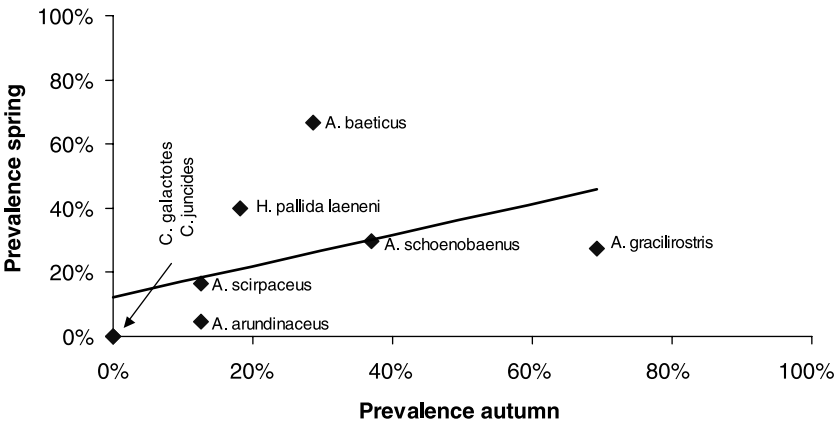

Fig. 1 Relationship between prevalence of *Haemoproteus* and *Plasmodium* in spring and autumn, for bird species in which at least four individuals were tested in both seasons.

species-specific prevalence levels. Of the 18 obtained haemosporidian lineages, 10 were found both in spring and autumn, seven in autumn only and one in spring only (Table 1).

Host specificity of the parasites

Two major clades, representing the two genera *Haemoproteus* and *Plasmodium*, were obtained when resolving the phylogenetic relationships between parasite lineages (Fig. 2), which is in accordance with Bensch *et al*. (2000). For both *Haemoproteus* and *Plasmodium*, we found lineages derived from both resident and migratory bird species. We found eight haemosporidian lineages infecting multiple host species, whereas the remaining 10 lineages were present in a single host species each (Table 2). Although most of the obtained sequences were unambiguous, six individuals gave sequences with one or more double peaks

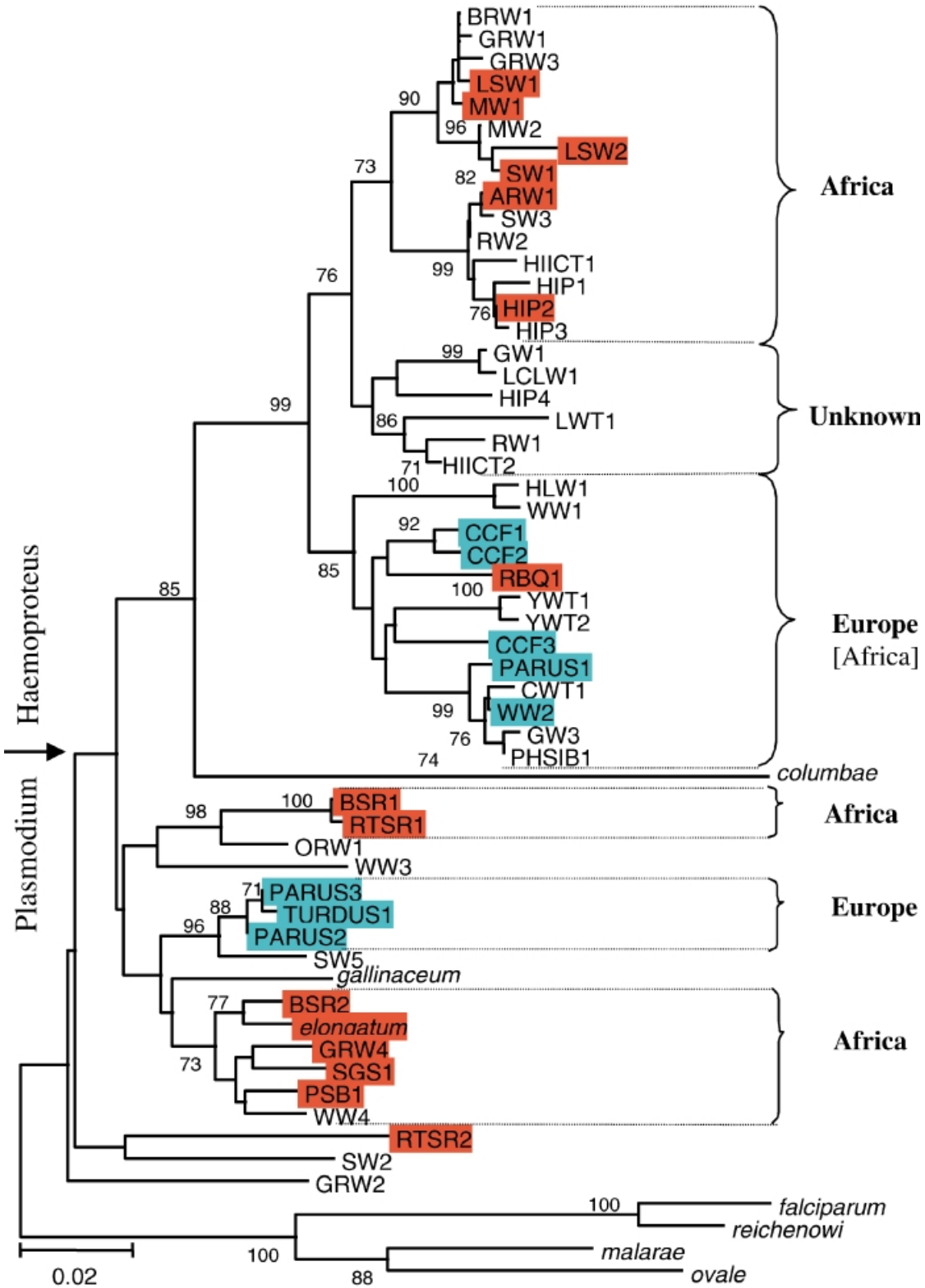

Fig. 2 A phylogenetic tree using the DNA sequence data of the partial cytochrome *b* gene (478 nucleotides) of *Haemoproteus* and *Plasmodium* (left) and the expected place of transmission (Africa or Europe). Lineages detected in African resident species are indicated in red and lineages detected in European resident species or juveniles of European migratory species in autumn in green. Lineages for which of transmission is unknown are uncoloured. For host species names of parasite lineages consult Appendix 1. The tree was constructed with the neighbour joining method with a Kimura 2-parameter distance. Bootstrap values (> 70%) are provided.

Table 2 Haemosporidian parasite lineages infecting multiple bird host species. The table consists of data from this study, and previously published (Bensch *et al*. 2000) or unpublished data (Bensch unpublished; species names in parenthesis)

Lineage	Bird species	Resident or migratory	*Haemoproteus* or *Plasmodium*
MW1	African reed warbler	Resident	*Haemoproteus*
	Eurasian reed warbler	Migrant	
	(Marsh warbler)	Migrant	
LSW1	Lesser swamp warbler	Resident	*Haemoproteus*
	Greater swamp warbler	Resident	
	Eurasian reed warbler	Migrant	
SW1	Eurasian reed warbler	Migrant	*Haemoproteus*
	Sedge warbler	Migrant	
	African reed warbler	Resident	
HIP2	Olivaceous warbler (*laeneni*)	Resident	*Haemoproteus*
	Olivaceous warbler (*opaca*)	Migrant	
ARW1	African reed warbler	Resident	*Haemoproteus*
	Eurasian reed warbler	Migrant	
GRW2	(Great reed warbler)	Migrant	*Plasmodium*
	Lesser swamp warbler	Resident	
GRW4	(Great reed warbler)	Migrant	*Plasmodium*
	Lesser swamp warbler	Resident	
	African reed warbler	Resident	
	Olivaceous warbler (*laeneni*)	Resident	
SGS1	(Sudan golden sparrow)	Resident	*Plasmodium*
	Eurasian reed warbler	Migrant	
	Sedge warbler	Migrant	
	(Lesser whitethroat)	Migrant	

in the electropherogram, suggesting that the host was infected with two haemosporidian lineages.

We found six lineages, five *Hameoproteus* and one *Plasmodium*, infecting different species of hosts within the same host genera and two lineages, both *Plasmodium*, infecting host species from different host genera (Table 2). One of the latter lineages was detected in species belonging to different families, the *Ploceidae* and the *Sylvidae* (Table 2).

Winter quarters and transmission

All of the eight lineages found in multiple host species were encountered in both resident and migrant bird species. Moreover, lineage LSW2 was obtained in the resident lesser swamp warbler *A. gracilirostris*. These nine lineages must hence have their transmission in Africa, i.e. the winter quarters of the migratory warblers. Figure 2 shows a phylogenetic tree of all available *Haemoproteus* and *Plasmodium* lineages, emphasizing those that have been encountered in resident bird species in Europe or in Africa. For *Haemoproteus*, one clade containing 16 lineages appears to be solely transmitted in Africa, as six have been detected in African resident species and none in European residents, whereas in the two other clades there were a mix of lineages of African, European or unknown transmission. For *Plasmodium*, two small clades imply winter transmission and one clade summer transmission.

Discussion

Cross-species transmission of parasites

We found eight lineages of *Haemoproteus* and *Plasmodium* in more than one host, involving both European migrant and African resident bird species. This clearly demonstrates that these blood parasites are less host specific than previously believed (Atkinson & van Riper 1991). An overwhelming majority of earlier studies used microscopy to study avian haemosporidians on blood smears. The taxonomy has been constructed using morphological traits, and also including the host species itself as supporting criteria (Atkinson & van Riper 1991). With a genetically based phylogeny, Bensch *et al*. (2000) demonstrated that the phylogenetic tree of the parasites poorly matched the phylogenetic tree of the hosts, suggesting that host shifts have occurred repeatedly in this host–parasite system. However, the only detected example of host sharing in that study was a *Haemoproteus* lineage coexisting both in great tits *Parus major* and blue tits *P. caeruleus* (Bensch *et al*. 2000). The results from our study demonstrate that host sharing is common both in *Haemoproteus* and *Plasmodium*, as 44% of the 18 detected parasite lineages were found in multiple host species. Host sharing is the crucial prerequisite for host shifts to occur, as previously was indicated by the mismatch between the phylogenetic trees of parasites and bird hosts (Bensch *et al*. 2000). *Plasmodium* species have

been considered less host specific than *Haemoproteus* (Atkinson & van Riper 1991). In this study, five of eight lineages of parasites infecting multiple host species were identified as *Haemoproteus* and three were *Plasmodium*, but the total number of *Haemoproteus* lineages was larger. All multihost *Haemoproteus* lineages were found in closely related host species, whereas we found one *Plasmodium* lineage that infected host species from different bird families. Our results imply that avian haemosporidian parasites are less host specific than previously thought, and the pattern suggests that host shifts occur relatively frequently in this system.

Timing and area of transmission

It is important to know the temporal pattern of transmission of parasites to their hosts, in particular to understand the evolutionary consequences they might incur on migratory birds. We applied our knowledge of when transmission events occur (presence in juvenile birds, shared strains between resident and migrant host species) to the phylogeny of parasite lineages (Fig. 2). In *Haemoproteus*, where the tree structure is highly supported, three major clades appear: (i) one with warblers (*Sylviidae*) in which transmission is strongly indicated to occur at winter quarters in Africa; (ii) one consisting of warbler species wintering in Asia and Africa, in which timing of transmission could not be inferred; (iii) and one with different host families (*Sylviidae*, *Fringillidae*, *Paridae*, *Motacillidae* and *Ploceidae*), in which most transmission events occur in the breeding areas in northern Europe. However, in this clade transmission undoubtedly occurs also in Africa as *Ploceidae*, a family in which all species are African residents, also carried parasites from these lineages. In *Plasmodium*, where the tree is not as well supported, two small clades with winter transmission and one with summer transmission were found, but further isolates are needed to enlarge the phylogenetic tree.

Additional support for parasite transmission taking place in Africa in winter in some clades comes from the difference in parasite prevalence between juveniles and adults in autumn. In several bird species, it has been observed that the prevalence of haemosporidians is higher in adult than in first year birds (e.g. Dale *et al*. 1996; Merilä & Andersson 1999; Sol *et al*. 2000). One explanation for this pattern is that older birds are more likely to have been exposed to vectors carrying the parasite, and thus to contract the disease. In migratory *Acrocephalus* species, adults exhibited higher parasite prevalence than juveniles, whereas there was no such age-related difference in resident *Acrocephalus* species. It should, however, be noted that the sample size was smaller for resident species. Juveniles of resident species generally spend much longer (during hatching, fledging, and early autumn) at the inferred area of parasite transmission (i.e. sub-Saharan Africa), and have therefore been exposed to the local fauna of parasites for a longer period than juveniles of migrant species that reach Africa from mid-autumn onwards.

Hence, we observed a mix of areas of transmission in several branches of the phylogenetic tree. This suggests that, in this host–parasite system, transmission of haemosporidian parasites to songbirds has arisen repeatedly in Africa and Europe.

Choice of wintering quarters and habitats

Parasite prevalence in hosts can differ, probably depending on the availability of the vectors (i.e. biting midges of the genus *Culicoides*), also on a rather small geographical scale (Sol *et al*. 2000; Freeman-Gallant *et al*. 2001) as well as in relation to nest height (Garvin & Remsen 1997). Given these small-scale geographical differences in probability of parasite transmission, a difference between bird species preferring wet or dry habitats seems plausible. If availability of vectors and parasites differs between regions, or within habitats, the risk of being infected will vary depending on where in the species' winter range, and in what habitat, a migratory bird overwinters. In our study, there was a large difference in parasite prevalence between bird taxa. Members of the two sister taxa *Acrocephalus* and *Hippolais* were infected more frequently than other studied groups. *Acrocephalus* have a strong association with water habitats, as 98% (n = 1347) of these were trapped at the wet locality, whereas *Hippolais* was slightly more likely to be trapped at the inland locality (63% trapped at the inland locality; n = 308). The other studied taxa were sampled in rather small numbers, but at least for *Cisticola* it appears that *Haemoproteus* and *Plasmodium* are either absent, or present only in very small numbers, in the investigated population. If parasitemia have effects on host fitness, as is likely to be the case for haemosporidian blood parasites (e.g. Atkinson & van Riper 1991; Richner *et al*. 1995; Nordling *et al*. 1998; Merilä & Andersson 1999), there should be selection towards choosing a winter site with low prevalence of avian haemosporidian parasites. In our study, we found six blood parasite lineages that infected multiple host species of the same genera, e.g. lineages MW1, LSW1, SW1, ARW1, GRW2 were found in *Acrocephalus* warblers and HIP2 in *Hippolais* warblers. Hence, from the point of avoiding parasites it could be advantageous for migratory species to avoid wintering areas with high densities of closely related resident species. This, however, may have to be traded-off against finding suitable habitats for stopover and wintering, because closely related species are likely to have rather similar habitat preferences.

Cost of migration

The cost of migration in birds has most often been evaluated in terms of energy expenditure (Alerstam & Lindström 1990) and predation risk (e.g. Lindström 1989; review over fat load and risk of predation), but may also involve moult patterns (Svensson & Hedenström 1999), wing morphology (Marchetti *et al*. 1995), competition at the wintering grounds (Price 1981; Rabol 1987; Jones *et al*. 1996) and competition for preferred breeding sites in spring (e.g. Bensch & Hasselquist 1991; Hasselquist 1998).

In earlier studies of avian haemosporidian parasites in Europe and North America, the prevailing hypothesis has been that hosts become infected during the breeding season in temperate regions (e.g. Garvin & Remsen 1997). If this was true, there would be very limited, if any, role for parasites as a factor contributing to 'cost of migration'. Our results challenge this view. We found several lines of evidence supporting the view that blood parasite infection can be a potential cost of migration. First, in the investigated species of songbirds, many lineages of avian haemosporidian parasites, both of *Haemoproteus* and *Plasmodium*, seemed to be transmitted in the African wintering areas, as we found several cases in which resident and migrant bird species shared the same blood parasite lineages. Data on prevalence and intensity of avian haemosporidians in juvenile and adult great reed warblers studied at their breeding sites in Sweden also strongly suggest that transmission of avian haemosporidian parasites does not take place at the breeding grounds (Ö. Östman, D. Hasselquist, S. Bensch unpublished). Second, cross-species transmission of avian haemosporidian parasites seems to be rather common, both for *Haemoproteus* and *Plasmodium*. In particular, we found that closely related bird species of migratory and resident origin shared the same blood parasite lineages. This is an important observation as it implies that African resident birds can act as reservoirs for blood parasites which then can infect migrant birds immediately when they reach their tropical wintering areas. Hence, there will be no time lag between arrival of the first migrant bird at a wintering site and the presence of vectors ready to transmit the parasites.

These two factors, i.e. parasite transmission occurring at wintering grounds and cross-species transmission of parasites between resident and migratory birds, clearly support the hypothesis that blood parasites can be a factor affecting cost of migration. Together with observations that parasite prevalence can differ considerably between nearby sites (Merilä *et al*. 1995; Sol *et al*. 2000; Freeman-Gallant *et al*. 2001) and in different habitats (this study), our data suggest that risk of blood parasite infection should be considered more carefully in the evolution of migration and patterns of winter distribution in migrating birds.

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Appendix I

Names of *Haemoproteus* and *Plasmodium* lineages and first encountered host species

Lineages	Host species	Lineages	Host species
YWT1, YWT2	*Motacilla flava*	LCLW1	*P. occipitalis*
BRW1	*Acrocephalus griseldis*	HLW1	*P. humeii*
LSW1, LSW2	*A. gracilirostris*	PHSIB1	*P. sibilatrix*
GRW1, GRW2, GRW3, GRW4	*A. arundinaceus*	CWT1	*Sylvia communis*
ORW1	*A. orientalis*	LWT1, LWT2	*S. curruca*
MW1, MW2	*A. palustris*	PARUS1, PARUS2, PARUS3	*Parus major*
SW1, SW2, SW3, SW5	*A. schoenobaenus*	TURDUS1	*Turdus philomelos*
ARW1	*A. baeticus*	BSR1, BSR2	*Cercotrichas podobe*
RW1, RW2	*A. scirpaceus*	RTSR1, RTSR2	*C. galactotes*
HIICT1, HIICT2	*Hippolais icterina*	RBQ1	*Quela quela*
HIP1, HIP2, HIP3, HIP4	*H. opaca*	CCF1, CCF2, CCF3	*Fringilla coelebs*
GW1, GW3	*Phylloscopus trochiloides*	SGS1	*Passer luteus*
WW1, WW2, WW3, WW4	*P. trochilus*	PSB1	*Anthreptes platurus*