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Haemosporidian parasites of a European passerine wintering in South Asia: diversity, mixed infections and effect on host condition

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Abstract We studied haemosporidian parasites in the scarlet rosefinch *Carpodacus erythrinus* in a small isolated semicolony during an eight-year period using molecular methods of parasite detection. The scarlet rosefinch is an interesting model of parasite host species. It winters in South Asia which represents a rare exception among European passerines. Males express yellow to red carotenoid-based plumage ornament which is a good predictor of male reproductive success. In 240 blood samples originating from 199 adult individuals, the total parasite prevalence reached 60 %. Prevalence varied among years from 36 to 81 % in *Haemoproteus*, 8 to 22 % in *Plasmodium*, and 0 to 14 % in *Leucocytozoon*. Twenty parasite lineages were detected (*Haemoproteus*: 5 lineages, *Plasmodium*: 10 lineages, and *Leucocytozoon*: 5 lineages). Among them, the *Haemoproteus* ROFI2 lineage, which is a host-specific parasite lineage of the scarlet rosefinch, was the most frequently found. Parasite lineages showed varying degree of lineage specificity. While *Haemoproteus* lineages detected in the scarlet rosefinch have relatively narrow host breadth restricted mainly to Fringillidae family, *Leucocytozoon* and *Plasmodium* lineages generally showed wider host range. The presence of some parasite lineages hitherto detected in sedentary European passerines (SISKIN1, CCF3, BT2) or in *Culicoides* biting midges at the same locality (ROFI1) suggest local transmission. On the contrary, lineages LK05 and FANTAIL1 that were previously reported exclusively from Asian hosts imply parasite transmission at the scarlet rosefinch wintering sites in South Asia. Mixed infections were found in 17 % of infected samples and comprised mainly the most frequent lineages. The pattern of concomitant infections seemed to be rather random and matched expected levels based on lineage frequencies. Between-year comparisons revealed that in a majority of the repeatedly captured individual hosts the infection status remained unchanged (individuals stayed uninfected or possessed the same parasite lineages). However, 16 gains and 8 losses of lineages were also reported. We have not found any effect of haemosporidians on male carotenoid ornament expression or host body mass.

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Introduction

Haemosporidians, frequent blood parasites of birds transmitted by blood-sucking Dipterian vectors, are diverse group comprising more than 200 described species classified into several genera: *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, which can cause bird disorders (Bennett et al. 1993). Molecular methods of parasite detection (Bensch et al. 2000; Waldenström et al. 2002; Richard et al. 2002; Fallon et al. 2003; Hellgren et al. 2004) started exponential grow of published studies covering various aspects of

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haemosporidian diversity, biology, host specificity, and effect on host fitness. Despite this extensive effort, our current view on many aspects of the host–parasite relationships (including parasite lineage specificity and the effect of parasites on host condition) has remained unclear.

Haemosporidian cytochrome *b* lineages which were shown to represent distinct evolutionary entities (Bensch et al. 2004, Valkiūnas et al. 2008a, Valkiūnas et al. 2008b) differ considerably in avian-host specificity. While *Plasmodium* lineages often develop in a large range of phylogenetically unrelated hosts (Waldenström et al. 2002; Križanauskienė et al. 2006; Hellgren et al. 2007; Beadell et al. 2009) and are able to spread between continents via frequent shifts from resident to migratory hosts (Hellgren et al. 2007), *Haemoproteus* lineages tend to show narrower host specificity (Bensch et al. 2009). Moreover, large variability in host specificity exists even among members of each genus. *Plasmodium* lineage SGS1 (assigned to *Plasmodium relictum* morphospecies) infecting almost 50 host species classified into several bird orders and some *Haemoproteus* or *Plasmodium* lineages found in single host species (Bensch et al. 2009) can serve as examples of the extreme limits in parasite–host specialization variability. Typically, Haemosporidian lineage spectra found in a particular host species consist of only few abundant parasite lineages and an array of infrequent lineages. These rare lineages may sometimes represent accidental transmissions into new hosts that cannot result in full development of the parasite and thus form a dead end in the parasite cycle (Valkiūnas et al. 2009a, Valkiūnas 2011). Since the rare infections are frequently transmitted to migratory birds at their wintering grounds where the regular local resident hosts may be known, lineage identity can provide interesting insights into the migratory host biology, especially in species in which the migration pathways are poorly documented.

Experiments performed under controlled laboratory conditions using mainly *Plasmodium* lineages repeatedly showed important pathogenic impact of haemosporidians on their avian hosts (Valkiūnas et al. 2006; Zehtindjiev et al. 2008; Palinauskas et al. 2011). However, the situation in nature seems to be more complex. While some studies demonstrated a significant effect of these haematobious parasites on bird fitness and/or condition (e.g. Seutin 1994; Merila and Sheldon 1999; Merino et al. 2000, Hõrak et al. 2001; Marzal et al. 2005, Westerdahl et al. 2005; Kilpatrick et al. 2006, Knowles et al. 2010), other studies failed to find any effect of this kind (e.g. Dale et al. 1996; Gonzalez et al. 1999; Votýpka et al. 2003; Deviche et al. 2005; Gibb et al. 2005; Bensch et al. 2007). This incongruity probably stems from the following reasons: (1) wild birds usually exhibit low levels of parasitemia suggesting chronic phase of infection which can only slightly affect the host (Valkiūnas 2005, Asghar et al. 2011), (2) while the parasite transmission, which is followed by acute phase of disease, frequently occurs at wintering grounds, host fitness or condition are measured mostly during the breeding season (Bensch et al. 2007), (3) parasites may be absent in peripheral blood in certain stages of their development and, thus, their detection using blood samples may be problematic (Zehtindjiev et al. 2008), (4) mixed infections which complicate the analyses are frequent (Marzal et al. 2008), and (5) studies may be biased by lower probability of sampling infected then uninfected birds (Valkiūnas 2005).

In our study, we have focused on investigation of haemosporidian parasites in a small community of scarlet rosefinch (*Carpodacus erythrinus*) breeding in the Czech Republic. In eight successive years, we trapped and sampled nearly all individuals at the breeding ground. Scarlet rosefinch is a small, semi-colonial, sexually dimorphic cardueline finch with delayed plumage maturation in males (Stjernberg 1979). Species breeding range spans from central Europe to Kamchatka and covers large area of the Palaearctic temperate zone. In the Czech Republic, the scarlet rosefinch breeds at the westernmost limit of its distribution which has been established by recent expansion of the species breeding range (Cramp et al. 1994). The scarlet rosefinch represents an interesting model of a haemosporidian host species for the following two reasons: (1) it is sexually dichromatic, with yellow to red carotenoid-based plumage of males signaling condition and male reproductive success (Albrecht et al. 2007, Vinkler et al. 2012). Hence, the male quality can be easily assessed, and the influence of parasites on male sexual traits can be studied. (2) Avian malaria research is strongly biased towards European–African and American migratory system. Scarlet rosefinch wintering grounds are located in South Asia which represents a rare exception among passerines breeding in Europe. Among birds breeding in Europe and wintering in South Asia, the paddyfield warbler *Acrocephalus agricola* has been the only species studied extensively for avian malaria using molecular methods (Zehtindjiev et al. 2009). Haemosporidian parasites in the scarlet rosefinch have been so far studied only marginally within the framework of comparative studies dealing with limited number of rosefinch individuals (Beadell et al. 2006, Križanauskienė et al 2006; Hellgren et al. 2007, Pérez-Tris et al. 2007). Four haemosporidian lineages have been hitherto detected in the scarlet rosefinch: *Haemoproteus* ROFI1 and ROFI2, and *Plasmodium* BT7 a SGS1. In our study population, we previously described the impact of *Haemoproteus* infection (detected by microscopy) on individual haematological state (Vinkler et al. 2010). In the present study, we used molecular methods to detect and precisely identify haemosporidian lineages in the same breeding population, estimate parasite prevalence and its temporal variation, reveal mixed infections, and examine the impact of infections on host body mass and male ornamentation.

Material and methods

Fieldwork

As the locality and field procedures were described in detail elsewhere (Albrecht 2004; Albrecht et al. 2007 and 2009; Vinkler et al. 2012), we mention here both only briefly. Rosefinches were studied from May to July during the years 2001–2008 at an isolated patch of wet shrubby meadow situated in the Vltava river valley (48°49' N; 13°56' E) in the Bohemia Forest National Park in Doudlebia, Czech Republic. Since the vegetation suitable for rosefinch nesting was searched systematically several times at the locality every year during the egg-laying period, almost all nests have been detected. Adults were mist-netted upon arrival to the locality or during the nest provisioning phase. Caught birds were transported into a field laboratory where basic measurements were taken (weight, tarsus length) and small amount of blood was obtained via brachial venipuncture and stored in ethanol. Breast patch colour (as the most important male ornamentation trait in this species) of males in their third year or older was measured using digital photography and subsequent computer analyses (all described in Albrecht et al. 2009). Shortly, photographs were taken under standard conditions and colour and grey charts were used to further standardize the measurements of colours. Hue, saturation and lightness of carotenoid ornament were measured (HSB colour space) using ADOBE PHOTOSHOP software (Adobe Systems Inc., San Jose, California). We also collected 17 individual blood samples of 13 other bird species trapped accidentally at the study plot (Appendix 1).

Parasite detection

All blood samples were dried in the laboratory and DNA was extracted using DNeasy® Tissue Kit (Qiagen). Presence and quality of the extracted host (Rosefinch) DNA was inspected by spectrophotometer NanoDrop® ND-1000 (Isogen Life Science) and by control PCRs using mitochondrial DNA (control region and ND2) primers (Pavlova et al. 2005) followed by agarose gel electrophoresis. Parasites were detected following the nested PCR protocol described in Hellgren et al. 2004 (see also Synek et al. 2013 for details), which enables to distinguish *Plasmodium* or *Haemoproteus* infections from *Leucocytozoon* ones using genera-specific nested primers. Positive samples were sequenced using primers HaemF (designed for *Plasmodium* or *Haemoproteus*) or HaemFL (designed for *Leucocytozoon*) (Hellgren et al. 2004). Parasite lineages were identified and classified according to MalAvi database (Bensch et al. 2009). All new haplotypes differing by one or more substitutions from the sequences deposited in GenBank were sequenced also from the 3′-end with primers HaemR2 and HaemR2L designed for *Plasmodium* or *Haemoproteus* and *Leucocytozoon* respectively. Since the two previously described haemosporidian lineages found in the scarlet rosefinch were named ROFI1 and ROFI2 (using contraction of the word rosefinch), we followed this habit and started the names of the new lineages with ROFI followed by numbers from 3 to 7 as recommended by Pérez-Tris et al. (2007). Sequences of the new lineages were deposited in GenBank (Accession numbers from JX556907 to JX556911).

The basic protocol used (Hellgren et al. 2004) enables simple detection of mixed infection of parasite lineages belonging to different genera (infection by a *Leucocytozoon* lineage and concurrently by *Plasmodium* or *Haemoproteus* lineage). However, mixed infections of *Haemoproteus* and *Plasmodium* lineages or mixed infections of single genus lineages require additional treatment (Loiseau et al. 2008; Marzal et al. 2008). Two strategies were adopted to distinguish these infections: (1) we checked carefully chromatograms for double peaks and (2) we designed lineage-specific primers (Table 1). PCR product of the initial PCR from standard protocol (Hellgren et al. 2004) was used as a template for nested amplifications with lineage-specific primers. Thermal conditions were the same as for the nested PCR in the standard protocol (Hellgren et al. 2004). In each examined sample, presence of the PCR product was verified using an agarose gel. The lineage-specific-primers strategy allowed us to identify mixed infections of *Plasmodium* and *Haemoproteus* lineages and also to detect mixed infections of the most common *Haemoproteus* lineage ROFI2 with other *Haemoproteus* lineages.\

Statistic analysis and other computations

We used generalized linear models (GLM) with identity link function (assuming normal distribution of dependent variables) for statistical evaluation of host–parasite interactions. Full models with reasonable two-way interactions involving the sex of the birds (where both sexes were treated) were fitted. Simplification of full models started with removing interactions and then the main effect (if they were not part of significant interaction). Hence, model terms were removed gradually, one by one, and models with and without the term of interest were compared using likelihood-ratio tests and F statistics (Crawley 2007). Presented are minimal adequate models (MAM) that means model with all members significant ($\alpha<0.05$). Each individual bird was used only once for the analysis (for those who were captured repeatedly, we used the infection status and other data from the first year when an individual was investigated). Analyses were made in R 2.14.2 software (Mathsoft 2011).

Table 1 Lineage-specific primers

Primer name	Sequence (from 5′ to 3′)	Product size (bp)	Parasite lineage(s) amplified
SelPlasF	CATGCAACWGGTGCWTCATT	271	*Plasmodium* lineages
SelPlasR	TTTTTAAGGTTGGGTCACTTACAAG		
SelHaemF	ATTGTTACYGCTTTYATGGGTTA	150	*Haemoproteus* lineages
SelHaemR	TCTTTTTAAAGTTGGATCACTWATAGT		
SelH62F	ATATGCATGCTACTGGAGCTA	477	*Haemoproteus* ROFI2 Lineage
SelH62R	AATAAACTTTGTGCTAAAAATATA		
SelH392F	TGCTACCGGTGCTACATTTG	471	*Haemoproteus* lineages CCF3, SISKIN1, ROFI1, ROFI3
SelH392R	AATAAACTTTGTGCTAGAAATAGG		

Results

Two hundred forty blood samples originating from 199 adult scarlet rosefinch individuals (some birds were captured repeatedly in different years) were analysed. We detected at least one haemosporidian lineage in 145 (60 %) samples. The total number of infections was even higher due to mixed infections (Table 2). Sequencing revealed 20 parasite lineages (Table 2) belonging to three haemosporidian genera: *Haemoproteus* (5 lineages), *Plasmodium* (10 lineages) and *Leucocytozoon* (5 lineages). Five of these lineages were newly identified and these differed by one to eight substitutions from the lineages described previously (Table 3). Individual lineages varied considerably in their prevalence: from dominant ROFI2 which was found in almost half of the samples analysed (112 positive samples, 47 %) to eight rare lineages in which each was detected in a single sample. Apart from ROFI2, only two lineages (SGS1 and BT2) exceeded the level of 5 % prevalence (both lineages 5.4 %). Hence, the pattern of

Table 2 Haemosporidian lineages found in the scarlet rosefinch. Number of infected individuals is given for particular lineage and year

Lineage/year (no. of samples)	2001 (28)	2002 (24)	2003 (21)	2004 (22)	2005 (37)	2006 (34)	2007 (37)	2008 (37)	Total (240)
Haemoproteus									
ROFI2	10	11	15	15	14	13	22	12	112
CCF3		1	1	1	1				4
SISKIN1				1					1
ROFI1			1					2	3
ROFI3					1		1		2
Plasmodium									
SGS1	2	1	1	2	1	2	1	3	13
PADOM02					2	1	2		5
WW3	1	1			1	1		1	5
ROFI4		1							1
TURDUS1								1	1
ROFI5								1	1
BT6								2	2
FANTAIL01		1	1						2
LK05						1			1
BT8					1				1
Leucocytozoon									
BT2	2	1	1	1		3	4	1	13
BT5		1							1
EMSPO05						1			1
ROFI6				1				1	2
ROFI7							1	1	2
Total	15	18	20	21	21	22	31	25	173

Table 3 New lineages

New lineage	Closest formerly described lineage	Closest lineage host(s)	Difference between lineages
Haemoproteus ROFI3	DENPEN02	*Dendroica pensylvanica*[a]	3 substitutions (0.6 %)
Plasmodium ROFI4	MOALB02	*Motacilla alba*[b]	1 substitution (0.2 %)
		Acrocephalus orientalis[b]	
Plasmodium ROFI5	MYITYR01	*Myiarchus tyrannulus*[b]	6 substitution (1.2 %)
Leucocytozoon ROFI6	EMSPO04	*Emberiza spodocephala*[c]	3 substitutions (0.6 %)
	EMSPO05		
Leucocytozoon ROFI7	SILUT01	*Sicalis luteola*[d]	8 substitutions (1.7 %)

[a] Ricklefs and Fallon 2002; Outlaw and Ricklefs 2009

[b] Beadell et al. 2006

[c] Palinauskas et al. unpublished

[d] Merino et al. 2008

lineage prevalence is markedly right-skewed. Parasite prevalence varied among years from 36 to 81 % in *Haemoproteus*, 8 to 22 % in *Plasmodium*, and 0 to 14 % in *Leucocytozoon* (Fig. 1).

While single-parasite lineage was found in 120 blood samples (83 % of positive samples), mixed infections were detected in 25 samples (17 %). Two lineages were found in most samples showing mixed infections (22 samples); however, three different lineages (one *Haemoproteus*, one *Plasmodium*, and one *Leucocytozoon* lineage) were found in three samples corresponding to three different birds (two females and one male). Mixed infection usually comprised the most frequent lineages (ROFI2 96 % of mixed infections, SGS1 24 %, BT2 24 %). Co-occurrence of lineages in mixed infections matched closely expected values calculated from lineage prevalence (Table 4). Expected total number of concomitant infections (cases when particular lineage shares its host with other lineage) did not differ significantly from observed value (expected 29, observed 28).

Thirty birds were trapped repeatedly (41 retraps) in consecutive years. The interval between repeated trappings of a particular individual spanned from 1 to 3 years. Four birds which occurred at locality in two different years remained uninfected. Three of them were re-trapped after two years (which suggests they bred one year outside our study plot). It means they were exposed to potential transmission for at least three breeding and wintering seasons. Apart from these uninfected birds, between-year comparisons revealed that in 20 infected individuals the infection status remained unchanged (they harboured the same lineages). However, we also detected 16 gains and 8 losses of lineages in host bloodstream. Losses involved lineages of all three genera (five lineage losses of *Haemoproteus*; one *Plasmodium*, two *Leucocytozoon*). Losses and gains also comprised lineage replacements: one ROFI2 loss was accompanied by gain of BT2, once FANTAIL01 was replaced by ROFI2, and one individual possessing originally ROFI2, BT2, and SGS1 was negative for ROFI2, BT2 and positive for SGS1 and ROFI1 in the next year.

Close inspection of the scarlet rosefinch lineages in the MalAvi database (Bensch et al. 2009) showed varying degree of lineage specificity (Appendix 2). Apart from five

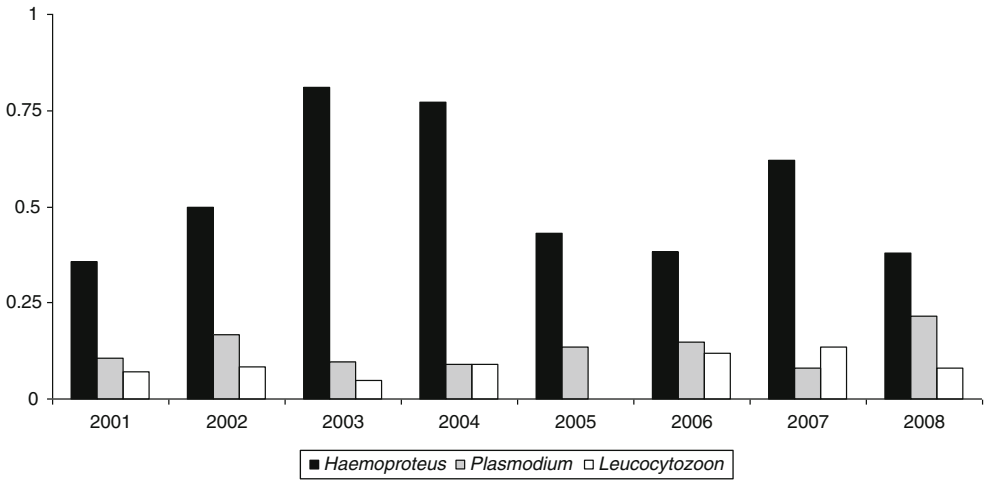

Fig. 1 Temporal changes of parasite prevalence

Table 4 Co-occurrence of parasite lineages. Number of mixed infections is given for particular lineage combination. Expected numbers based on lineage prevalence are given in brackets

	BT2	BT5	ROFI6	ROFI7	SGS1	PADOM02	WW3	ROFI4	TURDUS1	BT6	LK5	CCF3	ROFI3
ROFI2	6[a](6)	1[b](0)	2(0)	2[c](1)	5[a](6)	1(2)	1(2)	1[b](0)	1[c](0)	2(1)	1(0)	2(2)	2(1)
ROFI1					1(0)								

[a] Found also in mixed infection of ROFI2, BT2, and SGS1

[b] Found also in mixed infection of ROFI2, BT5, and ROFI4

[c] Found also in mixed infection of ROFI2, ROFI7, and TURDUS1

infrequent novel ROFI lineages, the most frequent lineage ROFI2 has not been reported in any other avian host species. While *Haemoproteus* lineages detected in our study were found exclusively in European passerines of the Fringillidae family (with the exception of SISKIN1 which was found in the house finch *Carpodacus mexicanus* and also the house sparrow *Passer domesticus* in the North America), *Plasmodium* and *Leucocytozoon* lineages show lower host specificity, including even non-passerine birds. They also often exhibit larger geographic distribution. However, *Plasmodium* lineage FANTAIL01 has been hitherto detected only in Australia and Southeast Asia, and *Leucocytozoon* EMSPO05 was known only from black-faced bunting *Emberiza spodocephala* from Russia.

Apart from lineages found in the scarlet rosefinch we detected in this study also six haemosporidian lineages in the other bird species trapped at the study plot (Appendix 1). Among them the *Leucocytozoon* BT2 lineage detected in three bird species was the only lineage shared with the scarlet rosefinch.

We found no association between presence of haemosporidian infections and the expression of condition dependent carotenoid-based colouration of males. The GLM model involving parasite occurrence (*Plasmodium*, *Leucocytozoon*, *Haemoproteus* and the total number of haemosporidian lines found in an individual) as explanatory variables for feather hue was not significant ($F_{4,93}$=0.92, P=0.45), the same was true for feather lightness ($F_{4,93}$=0.86, P=0.49) and saturation ($F_{4,93}$=0.68, P=0.61). In all cases the simplification suggested the null models as the best models.

In both males and females we furthermore evaluated potential associations between body mass (expressed as body weight/tarsus length) and parasite infection. The model involved haemosporidian genera and the total number of lineages, sex and sex interactions with haemosporidian occurrence and lineage numbers as an explanatory variable for body mass. The MAM only included sex as predictor for body mass ($F_{1,172}$=16.5, P<0.001), suggesting males were lighter for their tarsus length. However, no effect of haemosporidian infections on body mass was detectable (all P>0.15).

Discussion

We detected relatively high prevalence of haemosporidian parasites in the scarlet rosefinch, if compared to other published studies. However, since we studied only one locality our data may not reflect prevalence for the whole large species range. Prevalence differences have been detected among different populations of a single host species (Bentz et al. 2006, Ferrer et al. 2012) or within one population according to landscape features (Wood et al. 2007). Varying host exposure to insect vectors, which can be hardly assessed in migratory birds that can be infected at their wintering grounds, is probably the most important force determining the level of parasite prevalence (Yohannes et al. 2009). In our study, parasite prevalence varied considerably between years. Unfortunately the timescale was too short to reveal regular pattern of oscillations such were shown in the great reed warbler *Acrocephalus arundinaceus* (Bensch et al. 2007).

Since the migratory birds can be infected at breeding, wintering, and also migratory sites, they are supposed to host larger diversity of parasite lineages than the sedentary birds (Smith et al. 2004). This pattern has been demonstrated repeatedly in various species (e.g. Waldenström et al. 2002; Križanauskienė et al. 2006; Bensch et al. 2007; Hellgren et al. 2007; Ortego et al. 2008, Pérez-Tris et al. 2007). Relatively high number of parasite lineages (four *Haemoproteus* and five *Plasmodium* lineages) has been also detected in the paddyfield warbler (Zehtindjiev et al. 2009), which represents similar (European–South Asian) migration system as the scarlet rosefinch. The number of haemosporidian lineages detected in the scarlet rosefinch supports the view of larger parasite diversity in migrant species and contrasts sharply with only 5 lineages detected in the closely related sedentary house finch (*C. mexicanus*) (Kimura et al. 2006). However, it should be noted that most of the scarlet rosefinch lineages were extremely rare. With the exception of SIKIN1 lineage which was detected also in house sparrow (Marzal et al. 2011) all *Haemoproteus* lineages detected in the scarlet rosefinch have relatively narrow host breadth restricted to Fringillidae family. *Leucocytozoon* and *Plasmodium* lineages generally showed wider host range,

in some *Plasmodium* lineages even also comprising non-passerine species.

Tight co-evolution of parasite lineages and hosts that probably prevents parasites to infect sympatric closely related hosts has been reported repeatedly for avian malaria (Reullier et al. 2006, Pérrez-Tris et al. 2007). However, diversity of avian blood parasites was shown not to follow the classical trade-off-hypothesis suggesting that a generalist parasite infecting many hosts should occur at lower prevalence, whilst specialised parasite should rich higher prevalence in the host for which it is specialised (Hellgren et al. 2009). In contrast, Hellgren et al. (2009) showed that both *Haemoproteus* and *Plasmodium* lineages exhibiting the widest host range also occurred at the highest prevalence in a single host species. In concordance with these results, the most frequent *Leucocytozoon* and *Plasmodium* lineages detected in the present study had, indeed, the widest host ranges (*Plasmodium* SGS1 48 host species, *Leucocytozoon* BT2 8 host species). Nevertheless, the most frequent *Haemoproteus* lineage ROFI2, which is the most frequent haemosporidian lineage in the present study and was detected in the scarlet rosefinch also by Križanauskienė et al. (2006) and Bensch et al. (2009), has not been found in any other species, which indicates its strict host specificity.

Haemoproteus lineages SISKIN1 and CCF3 have been previously found only in European sedentary species, which suggests their local transmission to the scarlet rosefinch at the breeding site. We may also speculate that BT2 is transmitted during the breeding season, because it was present in other bird species at the study plot including the sedentary willow tit (*Poecile montanus*). Transmission during the scarlet rosefinch breeding can also be suggested for ROFI1 which was found in *Culicoides* biting midges at the same study plot in 2008 (Synek et al. 2013). On the other hand, the most frequent ROFI2 lineage was not found in haematophagous insects trapped at the same locality, which may suggest its transmission at wintering sites (Synek et al. 2013). Lineages that have been hitherto exclusively detected in Asian bird hosts (*Leucocytozoon* EMSPO05 known from *Emberiza spodocephala*, and *Plasmodium* FANTAIL01 detected in *Acridotheres tristis*, *Dendrocygna javanica* and *Rhipidura rufifrons*) correspond well with the assumed scarlet rosefinch wintering sites in South Asia. Since the EMSPO05 and FANTAIL01 have not been detected in any other European bird hosts, it is highly likely that scarlet rosefinches were infected by those lineages at their wintering sites. However, since avian malaria is far less studied in South Asia than in Europe and Africa we cannot exclude the possibility of winter transmission of other lineages that have been hitherto detected only in Europe and Africa.

Mixed infections of haemosporidian lineages have been repeatedly reported in avian hosts (e.g. Marzal et al. 2008; Valkiūnas et al. 2009b; Asghar et al. 2011; Zehtindjiev et al. 2012). Observed number of mixed infections in the present study closely matched the expected values based on prevalence of individual lineages, which may suggest random pattern of concomitant infections. However, it should be noted that the probability of detecting mixed infections strongly depends on the detection methods. For example, while detection based on manual inspection of electropherograms for double peaks suggested that concomitant infections were less frequent that expected in a long-term population study of great reed warblers (Bensch et al. 2007), closer inspection of partially overlapping dataset using specific primers and highly sensitive quantitative PCR showed on contrary significantly higher frequency of mixed infections than expected (Asghar et al. 2011). Even though we used both manual inspection of electropherograms and specific primer approach, we cannot fully exclude the possibility that we underestimated the frequency of mixed infections, especially in cases of low parasitemia in one of the lineages. On the other hand, in such a case the prevalence of individual lineages would be also underestimated in exactly the same way.

Infection status of birds trapped repeatedly in different years remained mostly unchanged. It may be interpreted either as evidence for a long-term survival of parasites in the host's bloodstream or as evidence for variability in individual host sensitivity to particular parasite lineages and consequent recurrent infections (Hasselquist et al. 2007). While the data showing long-term persistence of haemosporidian parasites in the chronic phase of their life cycle in avian hosts (Valkiūnas 2005, Zehtindjiev et al. 2008) support the former explanation, the known association between MHC alleles and resistance to avian malaria (reviewed in Westerdahl 2007) is consistent with the later explanation. On the other hand, relatively high rates of both *Haemoproteus* and *Plasmodium* lineage losses were also recorded in other bird species (*A. arundinaceus*: Bensch et al. 2007, Hasselquist et al. 2007; *Cyanistes caeruleus*: Knowles et al. 2011). In our study, the number of parasite lineage gains (16) twice exceeded lineage losses (8). However, since the *Haemoproteus* (five losses) and *Leucocytozoon* (two losses) lineages life cycles also involve stages in host internal organs, absence of gametocytes in the host bloodstream does not necessarily imply the full host recovery from the infection. Lineage replacements and the only one detected loss of *Plasmodium* lineage can be probably explained by very low parasitemia bellow the limits of our detection methods. In conclusion, we have not found any strong evidence of complete elimination of parasite lineages and hence our results are compatible with long-time persistence of parasites in bird hosts.

Carotenoid ornaments are generally considered as honest indicators of health (Lozano 1994, Badyaev and Hill 2000,

Vinkler and Albrecht 2010). In the present study, however, we have not found any effect of haemosporidian presence on male coloration and host body mass. In our previous study (Vinkler et al. 2010) based on microscopic examination of blood smears, we have shown that haemosporidian parasites may influence health in the scarlet rosefinch (measured as basophil ratio in peripheral blood). Nonetheless, the present lack of supportive evidence for the haemosporidian influence on body mass is in accordance with number of field and laboratory studies in birds (Sanz et al. 2002; Votýpka et al. 2003, Gibb et al. 2005; Deviche et al. 2005; Palinauskas et al. 2008). It is, thus, possible that the loss of weight due to blood parasites may be only temporary; occurring in acute phase of the infection and birds may recover to their original weight during the chronic phase (Valkiūnas 2005). Studies targeting the effect of haemosporidian parasites on carotenoid-based male coloration are rather scarce and inconsistent. While no effect of haemosporidian parasite presence on male coloration was found in redpoll (*Carduelis flammea*) Seutin (1994), negative correlation between haemosporidian presence and intensity of ornamentation was observed in yellowhammer (*Emberiza citrinella*) (Sundberg 1995). Surprisingly, also positive correlation of parasite presence and male carotenoid ornaments was reported in greenfinch (*Carduelis chloris*) (Merila and Sheldon 1999). Thus, it seems the effect of haemosporidians on carotenoid ornaments can be also rather complex and age dependent, as shown in blue tit (*C. caeruleus*) Hõrak et al. (2001). It should be also noted that the feather ornament of scarlet rosefinches can be influenced mainly during moulting completed during winter. We cannot exclude the possibility that the presence of parasites detected during the breeding season only weakly correlates with the bird infection status in winter. Moreover, acute winter infections that we were unable to detect during their chronic phase in spring may influence the host ornaments. However, this view remains unsupported by the fact that majority of birds trapped repeatedly in different years unchanged their infection status.

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Ethical standards This study was performed under certificate of competency according to §17 of the Act No. 246/1992 coll. on Protection Animals against Cruelty and comply with the current law of the Czech Republic.

Conflict of interest The authors declare that they have no conflict of interest.

Appendix 1

Table 5 Lineages found in other species trapped at the scarlet rosefinch locality

Host species	No. of individuals	*Haemoproteus*	*Leucocytozoon*
Common Treecreeper (*Certhia familiaris*)	1		
House Martin (*Delichon urbica*)	1		SYBOR8
Chaffinch (*Fringilla coelebs*)	1	CCF1	
Red-backed Shrike (*Lanius collurio*)	2	RB1	BT2
Bluethroat (*Luscinia svecica*)	1		BT2
Willow Tit (*Parus montanus*)	1		BT2
Common Chiffchaff (*Phylloscopus collybita*)	1		
Willow Warbler (*Phylloscopus trochilus*)	1		
Dunnock (*Prunella modularis*)	2	TURDUS2, RB1	
Garden Warbler (*Sylvia borin*)	2		
Common Whitethroat (*Sylvia communis*)	2	CWT3	
Lesser Whitethroat (*Sylvia curruca*)	1		
Blackbird (*Turdus merula*)	1	TURDUS2	

Appendix 2

Table 6 Lineage specificity. Other hosts of the scarlet rosefinch parasite lineages are listed according to MalAvi database. Six lineages found exclusively in the scarlet rosefinch are not included

Lineage		Host species	Geographic region
Haemoproteus	CCF3	*Fringilla coelebs*	Europe
	SISKIN1	*Carpodacus mexicanus*	Europe, North America
		Carduelis spinus	
		Loxia curvirostra	
		Passer domesticus	
	ROFI1	*Carpodacus erythrinus*	Europe
		Coccothraustes coccothraustes	
		Fringilla coelebs	
		Carduelis chloris	

Table 6 (continued)

Lineage		Host species	Geographic region
Plasmodium	SGS1	48 host species	Europe, Asia, Africa, New Zealand
	PADOM02	*Passer domesticus* *Anthus hodgsoni* *Corvus corone* *Luscinia svecica* *Motacilla flava* *Phasianus colchicus* *Emberiza citrinella* *Passer montanus*	Europe, South Asia
	WW3	*Andropadus virens* *Geothlypis trichas* *Quelea quelea* *Phylloscopus trochilus* *Spermophaga haematina* *Quelea quelea* *Phylloscopus trochilus* *Passer montanus* *Passer domesticus* *Cyanocorax yncas* *Luscinia svecica*	Europe, North America, Africa
	TURDUS1	18 host species	Europe, South Asia, Africa
	BT6	*Luscinia svecica*	Europe
	FANTAIL01	*Acridotheres tristis* *Dendrocygna javanica* *Rhipidura rufifrons*	Australia, South Asia
	LK05	*Falco namanni*	Europe
	BT8	*Luscinia svecica* *Ploceus* 2 species *Hypothymis azurea* *Estrilda amandava* *Copsychus* 2 species *Lophura punctuata*	Europe, Africa, South Asia
Leucocytozoon	BT2	*Lanius collurio* *Muscicapa striata* *Phoenicurus phoenicurus* *Phylloscopus trochilus* *Saxicola rubetra* *Sylvia atricapilla* *Sylvia borin* *Hippolais icterina*	Europe, Africa
	BT5	*Luscinia svecica* *Lanius collurio*	Europe
	EMSPO05	*Emberiza spodocephala*	Asia

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