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ORIGINAL ARTICLE

Characterization of haemosporidian infections in warblers and sparrows at south-western European reed beds

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Abstract The prevalence and intensity of avian haemosporian infections (genera *Haemoproteus* and *Plasmodium*) were investigated using molecular techniques and microscopy in nine passerine species at three Portuguese reed beds along a small latitudinal gradient. The effect of age, sex, season, site and year in the infection prevalence was evaluated for some of these host species. Of the sampled birds, 34.5% were infected, all with low level parasitemias. *Haemoproteus* spp. was only present in migrant species and was not locally transmitted, while *Plasmodium* spp. infected more species and reached a higher overall prevalence. Prevalence differed among bird species and was affected by different variables for each species: it was associated with age in the Reed Warbler *Acrocephalus scirpaceus*, with season in the Cetti's Warbler *Cettia cetti* and with year in the House Sparrow *Passer domesticus*. Site did not influence prevalence for any species at this small geographical scale. Reed Warbler adults had already migrated to Africa and contacted with two different parasite faunas, whereas juveniles had not, thereby explaining the importance of age to explain parasitemia in this species. For the resident Cetti's Warbler, prevalence varied significantly with season, perhaps due to lower food availability in autumn and winter, making birds weaker and more prone to infection.

Keywords Haemosporidiosis · Avian malaria · *Plasmodium* · *Haemoproteus* · Prevalence · Reed bed passerines · South-western Europe

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Zusammenfassung

Charakterisierung von Haemosporida Infektionen in Grasmücken und Sperlingen in Röhrichtgebieten in SW-Europa

Wir untersuchten die Prävalenz und die Intensität des Vorkommens von Haemosporidien (Gattungen *Haemoproteus* und *Plasmodium*) in neun Singvogelarten in drei Portugiesischen Röhrichtgebieten entlang eines geringen Nord-Süd Gradienten. Wir evaluierten die Effekte von Alter, Geschlecht, Jahreszeit, Gebiet und Jahr auf die Prävalenz der Infektionen für einige der Arten. 34,5% der beprobten Vögel waren infiziert, alle mit geringer Parasitämie. *Haemoproteus* ssp. war nur in Zugvogelarten

präsent und wurde nicht lokal übertragen, während *Plasmodium* ssp. mehrere Arten infizierte und insgesamt eine höhere Prävalenz erreichte. Die Prävalenz zwischen verschiedenen Arten war unterschiedlich hoch, und wurde durch für jede Art verschiedene Variablen beeinflusst: In Teichrohrsängern (*Acrocephalus scirpaceus*) war die Prävalenz mit dem Alter assoziiert, in Seidensängern (*Cetti cetti*) mit der Jahreszeit und in Haussperlingen (*Passer domesticus*) variierte die Prävalenz von Jahr zu Jahr. Auf dieser relativ kleinräumigen Skala hatte das Gebiet keinen Einfluss auf die Prävalenz, in keiner der Arten. Der Effekt des Alters auf die Prävalenz in Teichrohrsängern kann damit erklärt werden, dass adulte Teichrohrsänger bereits in Afrika waren und dort Kontakt mit einer anderen Parasiten Fauna hatten, während Jungtiere noch nicht dieser diversen Umgebung ausgesetzt waren. Für residente Seidensänger dagegen kann eine geringere Verfügbarkeit von Nahrungsmitteln im Herbst und Winter der Grund sein warum hier die Prävalenz saisonal variiert, welche die Vögel schwacher und damit empfänglicher für Infektionen macht.

Introduction

Parasites can reduce their host's fitness by weakening the host, causing pathologies and even modifying the host's behaviour, including decreasing its physical and feeding activity (Valkiunas 2005). They are a powerful selective force that influences and regulates hosts natural populations; as hosts fight back, both parasite infectivity and host resistance coevolve (Clayton and Moore 1997). Host–parasite interactions can be locally influenced by abiotic factors such as climate, season or habitat type, and by biotic effects such as host age or sex (Sol et al. 2003; Freeman-Gallant et al. 2001), causing each parasite's prevalence to vary throughout its distribution range. Local changes in the equilibrium of this system, such as climate changes or the introduction of new species, may originate the risk of disease outbreak (Atkinson and van Riper 1991).

Birds are often infected by blood parasites of the genera *Plasmodium* and *Haemoproteus* (Apicomplexa: Haemosporida). Although sometimes both genera are referred to as avian malaria parasites, strictly speaking only *Plasmodium* causes avian malaria. Both infect birds through the bite of an infected dipteran vector, which can be a mosquito in the case of *Plasmodium* spp. or a *Culicoides* midge or a hippoboscid fly for *Haemoproteus* spp. (Valkiunas 2005). After the transmission episode, there is a pre-patent period during which the parasites reach the blood cells, and then the infection's intensity in the blood stream (also called parasitemia) rises until it reaches a peak.

Among birds, the passerines can have relatively high prevalence of haemosporidians, although there are variations between host species and even between populations of different geographical areas (Valkiunas 2005). For example, House Sparrows *Passer domesticus* showed a prevalence of 47% *Plasmodium* spp. in one French population and of 75% *Plasmodium* spp. and 0.01% *Haemoproteus* spp. in another population (Bonneaud et al. 2006). These geographical differences may arise from the sites' differences in habitat conditions, bird community (presence of alternative host species), vector abundance and vector activity, which influence parasite transmission (Pérez-Tris and Bensch 2005a). Also, each parasite species can have its own transmission season (winter, summer or year-round), causing variation in prevalence throughout the year (Pérez-Tris and Bensch 2005b); if the parasite community of all sites is not the same, this will add to the differences between sites. In migrant species, the observed prevalences in a particular site are also influenced by the conditions and parasite faunas encountered during the whole migration cycle. Such is the case of the Great Reed Warbler *Acrocephalus arundinaceus*, that showed 10% *Haemoproteus* spp. and 10% *Plasmodium* spp. in Latvia, 21% *Haemoproteus* spp. in Germany (Bensch et al. 2000), 17% *Haemoproteus* spp. and 27% *Plasmodium* spp. in Sweden (Bensch et al. 2007), and 31% *Haemoproteus* spp. and 23% *Plasmodium* spp. in Bulgaria (Dimitrov et al. 2010).

Reed beds are relatively isolated patches of habitat, which can differ in their conditions and vector community, so they are expected to have a large geographic variation in their dynamics of parasite transmission. There is substantial information on haemosporidian infections in reed bed passerines, but this knowledge is not homogeneous across the breeding and the migration range. While a lot is known about the northern European populations (see all references in the paragraph above), there is little knowledge on their southern European breeding range (Fernandez et al. 2010; Merino et al. 1997, 2000).

This study aimed to find which factors are associated with haemoparasite prevalence in several passerine species. Microscopy and molecular techniques were used to detect *Haemoproteus* and *Plasmodium* spp. infections in two species of sparrows (family Passeridae) and seven species of migrant and resident Old World warblers (four families of the superfamily Sylvioidea) at three different Portuguese reed beds along a small latitudinal gradient. The prevalence was analysed according to site, season and host's characteristics for four of these bird species: the Cetti's Warbler *Cettia cetti*, the Reed Warbler *Acrocephalus scirpaceus*, the Tree Sparrow *Passer montanus* and the House Sparrow.

Methods

Study area

Samples were collected in three Portuguese wetlands close to the coast: Taipal (40°11′N, 8°41′W), Santo André (38°4′N, 8°48′W) and Vilamoura (37°04′N, 8°07′W), aligned along a latitude gradient of 370 km. Santo André is a brackish water coastal lagoon, while the other two are freshwater marshes. These sites' vast reed beds (*Phragmites australis*) house a wide variety of ducks, waders and other water birds, both resident and migratory. They are important breeding sites for resident passerines like the Cetti's Warbler and also breeding and refuelling areas for migrants such as the Reed and the Great Reed Warblers. The still waters of the three study sites are good breeding areas for mosquitoes, which are abundant from May to September (personal observation).

Field work

Passerines of nine species were sampled from March 2007 to November 2008 in all the sites. Three seasons were considered: breeding season (March–July), summer/autumn migration (August–September) and winter (October–January). The Eurasian Tree Sparrow, the House Sparrow and the Cetti's Warbler were residents, so could be sampled in all seasons; the Willow Warbler *Phylloscopus trochilus*, a passing migrant, was mostly sampled in autumn; the Common Chiffchaff *Phylloscopus collybita* winters in the study area, so was only captured during autumn and winter. Four other species breed in these marshes and are absent during winter, so were only found during the breeding season and autumn: the Reed and the Great Reed Warblers, the Savi's Warbler *Locustella luscinioides* and the Iberian Chiffchaff *Phylloscopus ibericus*.

Individuals were captured with mist nets, ringed, weighed, measured and then aged according to Svensson (1992). A blood sample (around 40 µl) was collected from the jugular or brachial veins using a 25- or 30-G needle, after which the birds were released. From that sample, a drop of blood was used to make a smear and the rest was stored in 96% ethanol for future DNA amplification. The smears were prepared according to Valkiunas (2005), air-dried and fixed as soon as they were dry in 96% ethanol for 3 min.

Laboratory work

Within 2 weeks from preparation, the smears were stained with a 10% solution of Giemsa's stain for 50 min (Valkiunas 2005). Smears were screened for parasites with a light microscope. First, the whole smear was examined under a ×400 magnification, and then random fields were screened for parasites under ×1,000 magnification until 20 fields (averaging 10,000 erythrocytes) had been observed. Infection intensity was considered to be the number of detected parasites per 10,000 screened erythrocytes.

Total DNA was extracted from the blood samples using a standard ammonium acetate protocol. Birds were sexed by a polymerase chain reaction (PCR) amplifying a CDH gene fragment, using the primers 0057F (3′-CGTCAATTT CCATTTCAGGTAAG-5′) and 002R (3′-TTATTGATCC ATCAAGTCTC-5′). The reaction products were run in 2% agarose gels for band visualisation and sexing of each sample. Success in this reaction also confirmed that the extracted DNA was in good enough condition to be amplified by PCR.

Infections were diagnosed using a nested PCR protocol developed by Waldenström et al. (2004), targeted at a portion of the parasite's mitochondrial cytochrome *b* gene. The used primers were specific for *Haemoproteus* and *Plasmodium* spp.: HaemNF/HaemNR2 (Waldenström et al. 2004) for the pre-amplification PCR, followed by HaemF/HaemR2 (Bensch et al. 2000) for the specific PCR. Each reaction had a total volume of 25 µl and included approximately 25 ng of genomic DNA, 1.5 mM $MgCl_2$, 2.5 µl of 10× PCR buffer II, 400 mM of each deoxynucleoside triphosphates, 0.6 mM of each primer, and 0.625 U of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA, USA). The thermal profile started with 3 min of denaturation at 94°C, followed by cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 45 s, and ended with an elongation step at 72°C for 10 min. The pre-amplification PCR ran for 20 cycles and the final PCR ran for 35 cycles (Waldenström et al. 2004). Final amplification products (479 bp) were run in a 2% agarose gel.

We controlled for contaminations by including a negative control per each 24 samples during extraction and a negative control (water) for each 8 samples during PCR. None of these controls ever showed amplification. Negative results were confirmed by a second nested PCR. Samples showing positive amplification were precipitated with ammonium acetate and ethanol and sequenced using primer HaemF. The obtained sequences were aligned and edited with BIOEDIT (Hall 1999) and compared with the sequences stored at GenBank and the MalAvi database (Bensch et al. 2009) for identification of the parasite's genus.

Statistical analysis

Birds were considered infected when they had a positive PCR or an infected smear [in the few cases (1.8%) in which PCR failed to detect infections that were confirmed in smears]. All the infected birds were considered to be infected by one single parasite lineage, even in the five cases when co-infections of parasites of different genera

were recorded by microscopy. The used PCR protocol frequently reads mixed infections of different haemosporidian species as if they were single infections, identifying only one of the present parasite lineages (Valkiunas et al. 2006a). Therefore, in the cases of co-infections by *Haemoproteus* and *Plasmodium* spp., the prevalence of one of these genera was underestimated, but the fraction of infected individuals of each host species and the overall intensity of infection were not altered by this procedure.

Prevalence of Haemoproteus and Plasmodium spp.

A log-linear analysis was performed to test the association of year, season and the sampling site with the presence or absence of infection (a variable that reflects prevalence, defined as infection status: 0 = bird not infected, H = bird infected with *Haemoproteus* spp. and P = bird infected with *Plasmodium* spp.). This analysis was carried out for the four most abundant bird species in the three study areas: Cetti′s Warbler, Reed Warbler, House Sparrow and Tree Sparrow.

The log-linear models were built using with STATISTICA 7 (Statsoft 2002) by a stepwise process. First, all *k*-factor interactions ($k = 1$, 2, etc.) between variables were tested simultaneously to find which order of interactions needed to be included to significantly explain the data. Then, the terms of that order or lower that significantly improved the model's fit were included one by one (Statsoft 2002). The fit of the models to the data was determined using maximum likelihood χ^2 tests (H_0: the model describes the variation in the data) and the accepted model for each host species was the least complex model that fitted the data.

Infection intensity

For the four host species with a larger sample (Table 1), a general linear regression model was built to relate infection intensity with age, sex, site, season, year and body condition. These models were built including all effects in STATISTICA 7.0 (Statsoft 2002). The dependant variable was the $\log_{10}$ (intensity + 1). An intensity value of 0.5 was attributed to infections undetected by smear observation but with a positive PCR (considering that these samples have infections weaker than 1 parasite per 10,000 erythrocytes). Age, sex, site and year were seen as categorical predictors. The body condition, a continuous variable, measured the variability in weight that was not explained by the animal's body size. This parameter was the standard residual obtained from a linear regression between weight and wing length for each species.

Results

Prevalence of *Haemoproteus* and *Plasmodium* spp.

A total of 1,057 birds from nine species were sampled, out of which 365 (34.5%) showed infections: 5.8% by *Haemoproteus* spp., 26.9% by *Plasmodium* spp. and 1.8% were not identified (corresponding to 19 infections detected in the smears, but which could not be amplified by PCR). Prevalence varied considerably between bird species, from 58.8% for the Cetti's Warbler to zero for the Common Chiffchaff (Table 1). There were significant differences between prevalence of infection in bird species of the same genus, except for *Passer* (for *Acrocephalus*, $\chi^2_1 = 8.02$, $P = 0.004$; for *Phylloscopus*, $\chi^2_2 = 5.94$, $P = 0.05$; but for *Passer*, $\chi^2_1 = 0.0004$, $P = 0.985$).

Within the whole sample of infected birds, the number of infections by *Plasmodium* (82.3%) was significantly higher that the number of birds infected by *Haemoproteus* spp. (17.7%, $\chi^2_1 = 154.9$, $P < 0.001$). Supplementary table S1 provides the complete list of all the lineages found in this study. The most prevalent lineage of parasites was SGS1, which caused 54.2% of all infections, including 91.7% of

Table 1 Total sample size and infection prevalence for the nine host species

Bird species	Sample size	Infected (%)	Parasite genus
Cetti's warbler, *Cettia cetti*	245	144 (58.8)	144 P
Great Reed Warbler, *Acrocephalus arundinaceus*	33	19 (57.6)	12 H, 7 P
Reed Warbler, *Acrocephalus scirpaceus*	387	128 (33.1)	46 H, 63 P, 19 unid
Savi's Warbler, *Locustella luscinioides*	45	7 (15.6)	1 H, 6 P
Common Chiffchaff, *Phylloscopus collybita*	116	0	–
Iberian Chiffchaff, *Phylloscopus ibericus*	27	1 (3.7)	1 P
Willow Warbler, *Phylloscopus trochilus*	36	2 (5.6)	2 H
House Sparrow, *Passer domesticus*	114	44 (38.6)	1 H, 43 P
Tree Sparrow, *Passer montanus*	54	20 (37.0)	20 P
Total	1,057	365 (34.5)	62 H, 284 P, 19 unid

Parasite genera are: *P Plasmodium*, *H Haemoproteus*, *unid* unidentified genus

Table 2 Best log-linear models reflecting the variables that influence infection status for each species

Host species	Influent variable	Variable's partial association	Final model's test of fit (max. lik. χ^2)	Effect of the selected variable in the infection status
Cetti's warbler	Season	$\chi^2 = 11.63$, $P = 0.003$	$\chi^2 = 23.34$, 23 *df*, $P = 0.441$	Prevalence (of *Plasmodium* spp.) is lower in the reproductive season, higher in winter
Reed warbler	Age	$\chi^2 = 36.06$, $P < 0.001$	$\chi^2 = 102.85$, 124 *df*, $P = 0.917$	Prevalence increased with age, both for *Plasmodium* spp. and for *Haemoproteus* spp.
House sparrow	Year	$\chi^2 = 7.497$, $P = 0.006$	$\chi^2 = 19.88$, 22 *df*, $P = 0.590$	Higher prevalence in 2007
Tree sparrow	None	–	$\chi^2 = 5.18$, 15 *df*, $P = 0.990$	None of the tested variables affected infection status

The tested variables were age, sex, season, site and year. Partial associations are computed by evaluating the gain of fit of the model that includes the corresponding interaction with the model that excludes it (Statsoft 2002)

infections in the Cetti's Warbler, 6.3% in the Reed Warbler, 81.8% in the House Sparrow and 95.0% in the Tree Sparrow.

Haemoproteus spp. was only present in migrant species ($\chi^2_1 = 18.64$, $P < 0.001$), with the single exception of one House Sparrow, a species known to spend limited time in the reed bed. Almost all the migrants infected with *Haemoproteus* spp. were adults; only two Willow Warblers sampled during migration in autumn 2007 were juveniles. Therefore, all three exceptions were birds that had certainly spent much time outside the study sites. This suggests that none of the *Haemoproteus* lineages was transmitted locally and that the infected birds probably acquired those parasites elsewhere. The *Plasmodium* genus was detected in resident species and/or in juveniles that were still attached to their birth reed bed, which proves local transmission of at least some lineages of this genus, such as SGS1.

The log-linear analyses suggested that different factors contribute to explain the infection status of each of the four tested bird species (Table 2). The prevalence in the Cetti's Warbler was affected by season, increasing from the reproductive season to autumn and maintaining the same higher levels of infection in winter (Fig. 1). This pattern was also present to some extent in the House Sparrow (Fig. 1). The prevalence in the Reed Warbler was affected by age (Fig. 2), increasing in adulthood both for *Plasmodium* spp. and for *Haemoproteus* spp. For the House Sparrow, prevalence was associated with the year, showing differences between study years. Tree Sparrows did not show an effect of any of the tested variables. Site and sex were unimportant for all species. The same variables were elected when these models were redone excluding *Haemoproteus* infections, since their transmission was not affected by local conditions.

Intensity of infections

A total of 772 smears were examined, revealing 122 infections (Fig. 3). For the same birds, PCR results show

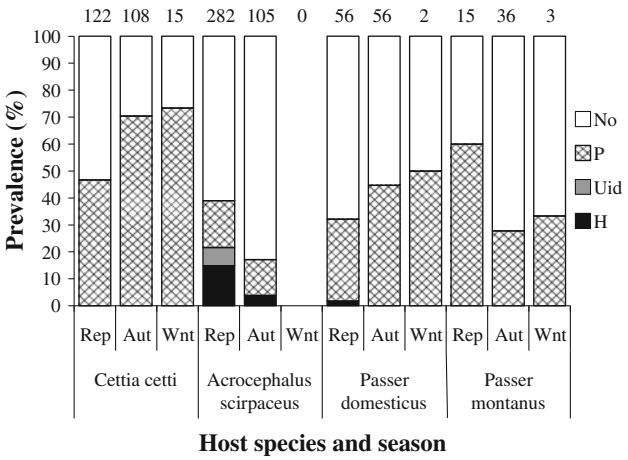

Fig. 1 Prevalence by season for the four species with a larger sample. Seasons are: Rep = reproductive season, Aut = autumn and Wnt = winter; infection states are: No = no infection, P = *Plasmodium*, Uid = unidentified genus, H = *Haemoproteus*. Sample size for each season and species is shown above each column. *A. scirpaceus* is absent from the study sites during winter

280 infections, suggesting that most of these parasitemias were lower than 1 parasite per 10,000 erythrocytes (i.e., below the threshold of detection by the used microscopy protocols). Infection intensity ranged from less than 1 to 291 parasites per 10,000 erythrocytes, which is considered a low level parasitemia (Valkiunas 2005; Zehtindjiev et al. 2008).

For the 280 infected birds, infection intensity was related with the genus of the parasite: it was significantly higher for *Haemoproteus* than for *Plasmodium* spp., both for each species and for the overall set of samples ($\chi^2_2 = 111.17$, $P < 0.001$). For each of the four analysed species, the general linear regression model built to explain infection intensity (not shown) included the same significant variable as the model that explained prevalence for that species. This was maintained when only *Plasmodium* spp. infections were considered.

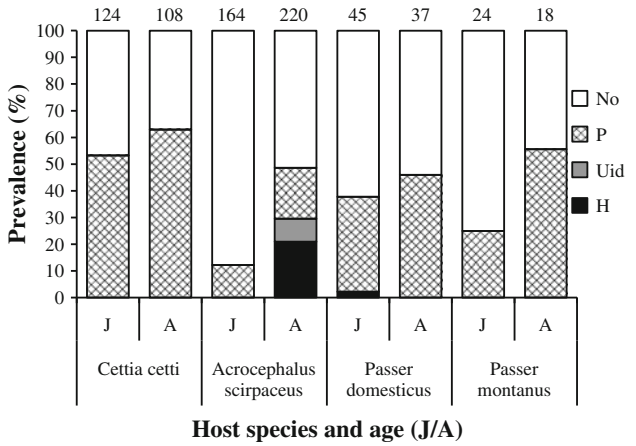

Fig. 2 Prevalence by age (*J* juveniles, *A* adults) for the four analysed species. Infection states are: *No* no infection, *P Plasmodium*, *Uid* unidentified genus, *H Haemoproteus*. Sample size for each season and species is shown above each *column*

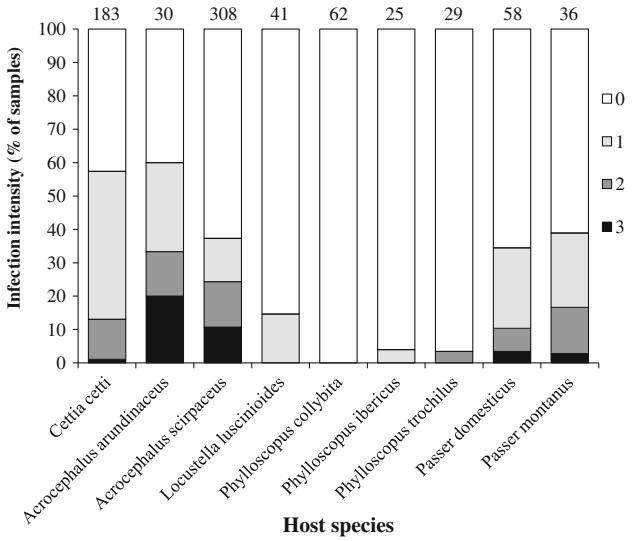

Fig. 3 Infection intensity in the nine bird species. *0* not infected, either by PCR or by smear observation. Infections that were detected by PCR are classed as: *1* <1 parasite/10,000 erythrocytes (infection detected by PCR, but not in smear); *2* 1–10 parasites/10,000 erythrocytes; *3* >10 parasites/10,000 erythrocytes. Sample size for each bird species is shown above its *column* (total sample size = 772)

Discussion

Prevalence

The overall prevalence found in the studied bird species (34.5%) was within the range of previous observations in warblers and sparrows in other areas, using similar detection techniques (Bonneaud et al. 2006 in France; Valkiunas et al. 2008 in Lithuania; Waldenström et al. 2002 in Nigeria). Prevalence in species of the same genus was significantly different for the warblers, but not for the sparrows. For birds of these genera, some studies have also found differences (Bensch et al. 2000; Dimitrov et al. 2010; Pérez-Tris et al. 2007; Waldenström et al. 2002), while others have not (Fernandez et al. 2010; Shurulinkov and Ilieva 2009). This indicates that prevalence is not phylogenetically determined and is probably more influenced by immune, physiological and ecological constraints.

There seems to be no transmission of *Haemoproteus* spp. in our study sites, although individuals infected elsewhere have active infections in their bloodstream. This agrees with the fact that the main known vectors of *Haemoproteus* spp., the biting midges (genus *Culicoides*, Ceratopogonidae), prefer forested habitats and seem to be absent from the studied reed beds (R. Ventim, unpublished data). Transmission of some *Haemoproteus* lineages seems to occur exclusively in Africa (Hellgren et al. 2007a; Waldenström et al. 2002), while some *Plasmodium* parasites such as SGS1, a lineage of *Plasmodium relictum* (Palinauskas et al. 2007), seem to adapt better to several different transmission conditions (Hellgren et al. 2007b), so can be transmitted locally as well as in Africa.

The log-linear analysis showed that the overall prevalence depends on different variables for each of the studied bird species. For the Reed Warbler, the only analysed migrant, age was the most important factor. This is because the juveniles have only been exposed to the local parasites, whereas adult individuals have already migrated and been exposed to the parasite faunas of both their wintering and reproductive sites, which should explain their higher overall prevalence. This was obvious for *Haemoproteus* parasites, but may also happen with some African-transmitted *Plasmodium* lineages (Hellgren et al. 2007b). The remaining species, all residents, showed different patterns of effects. This means that every host species has a different susceptibility to infection, which can be mediated by differences in their vector attraction, exposure behaviours, immune system's reactions to environmental changes, etc. Also, different birds can be infected by distinct lineages, each with its own transmission rhythms and patterns that can be masked when the overall prevalence is analysed.

An increase in prevalence during the reproduction season could be expected due to several possible causes: (1) an increase of vector populations because of good temperature and humidity conditions; (2) a reduced host immunocompetence due to reproduction stress and energy investment (Mendes et al. 2005; Schultz et al. 2010), making the birds more susceptible to be infected; and (3) the spring relapse of latent infections, associated with the previous factor (Atkinson and van Riper 1991; Valkiunas 2005; Schultz et al. 2010). However, the season's effect was only significant for the Cetti's Warbler, in which the prevalence increased strongly from spring to autumn. The Cetti's Warbler body condition dropped at the end of summer, at

the same time in which arthropod availability should have decreased in the reed beds. Therefore, the more stressful season of the year for resident birds might be autumn and winter, leaving them more immuno-depressed and prone to infections than reproductive stress during the breeding season. Mosquitoes are abundant in these areas until late summer (September); the infections contracted at that time should manifest during autumn (after passing the pre-patent period; Palinauskas et al. 2008) and were probably maintained during winter, while the birds' physical condition continued to be poor. This can explain why prevalence was higher during autumn and winter for resident bird species, even though there were a lot fewer mosquitoes in those seasons (R. Ventim, unpublished data). This tendency should be more obvious in more specialised species, like the Cetti's Warbler, and more subtle in resident generalists such as the House Sparrow, since generalists can also rely on other food sources.

The location did not influence prevalence patterns at this small geographic scale. The reed beds of our study are geographically isolated patches and therefore they were expected to have somewhat different communities of hosts and vectors, generating differences in the transmitted parasites (Pérez-Tris and Bensch 2005b). However, the bird communities in these patches are probably connected through the juvenile dispersal movements of their residents, stopping-over of migrants that have nested in different locations and the contact between migrant populations of different origins in their wintering grounds. These bird movements, together with similar vector communities, are apparently enough to make the parasite communities similar in all the studied sites. Other studies have found significant spatial differences in the prevalence in breeding populations of many bird species; however, those studies were conducted at a wider geographical scale (Bennett et al. 1995 in Scandinavia; Bensch and Akesson 2003 in Sweden; Merila et al. 1995 throughout Europe), compared different habitats (Loiseau et al. 2010 in Ghana) or compared sites at different altitudes (Shurulinkov and Ilieva 2009 in Bulgaria).

Intensity of infections

All the infected birds that were sampled had low level parasitemias, consistent with the chronic phase of infection (Valkiunas 2005; Zehtindjiev et al. 2008). Heavily infected birds, at the peak of their infection, are seldom sampled using mist nets because they should be weakly mobile or less active than healthy individuals (Valkiunas 2005). Because in practice this group of individuals is not covered by our sample, the actual prevalence of haemoparasites in the wild should be somewhat higher than what can be found using this sampling method. The parasitemia of *Haemoproteus* spp. infections was significantly higher than that of *Plasmodium* spp. This confirms the findings of Atkinson and van Riper (1991), even at the chronic stage of infection.

In the four analysed host species, the body condition was not related to infection intensity or to the presence/absence of infection. This agrees with other studies of experimental infections (Garvin et al. 2003; Valkiunas et al. 2006b) and natural infections in several species (Bennett et al. 1988; Edler et al. 2004; Schultz et al. 2010). However, this kind of relationship is hard to detect in wild animals because of the afore-mentioned under sampling of acutely infected birds, which are those that might show a depressed body condition (Valkiunas 2005).

This was the first study of haemosporidian transmission in SW Europe including a community of nine passerine species. Extensive sampling of this kind adds to the accumulated knowledge of avian malaria infections, helping to appreciate presence and prevalence differences between distinct geographic regions or environmental conditions.

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