Blood parasites prevalence of migrating passerines increases over the spring passage period

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Short title for page headings:

Parasite prevalence on spring passage

Word count= 3270

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Abstract

Whether long-distance animal migration facilitates or hampers pathogen transmission depends on how infections affect the routes and timing of migrating hosts. If an infection directly or indirectly impedes migratory flight capacity, infected individuals lag behind their uninfected conspecifics. Although such temporal segregation can limit parasite transmission and thus play an important role for host-parasite interactions, empirical evidence remains scarce.

Here we investigated haemosporidians – blood parasites commonly infecting birds – in four passerine species on spring passage and linked infection status to passage date. As a step towards identifying the mechanisms behind infection-related delays, we incorporated sets of individual, energetic, haematological and biometric variables into the analysis.

Haemosporidian prevalence virtually doubled between birds sampled at the beginning of the passage period with those sampled one month later. This indicates that infected individuals arrived later than uninfected individuals. Both the average prevalence and its increase over time varied among host species. Additionally, the leucocyte counts of infected birds were elevated, suggesting that immune response may require resources which could otherwise be allocated to migratory flights. However, infection status was not related to any other variable like of body mass, energy stores, sex, age and feather length. Yet regardless of the underlying mechanisms, infection-related differential timing might influence transmission and affect pathogen prevalence in wildlife populations year-round.

Keywords: Haemosporida, immune response, migration timing, spring passage, stopover

Background

Host migration is a mixed blessing for zoonotic pathogens (Altizer, Bartel & Han, 2011). Long-distance migratory hosts may encounter and accumulate more diverse parasites than residents (Figuerola & Green, 2000) and the elevated energetic demands of endurance locomotion might suppress a host's immune system (Norris & Evans, 2000), making migrants more susceptible to infections (Møller & Erritzøe, 1998).

Conversely, migrations may hamper transmission and reduce prevalence in host populations, e.g. when hosts abandon pathogen-rich sites to later return into pathogen-poor habitats ("migratory escape"; Altizer *et al.*, 2011). Likewise prevalence declines when infections lead to elevated mortality on migration ("migratory culling"; Altizer *et al.*, 2011). Furthermore, if migration propensity or routes differ between infected and uninfected hosts e.g. between immune-naïve juveniles and pathogen-experienced adults (Krkošek *et al.*, 2007), this also reduces transmission risk ("migratory allopatry"; Johns & Shaw, 2016). In addition to these spatial differences in migration patterns, there might also be a temporal separation of migration waves on the same route (henceforth referred to as "migratory allochrony"). Such a temporal separation might result when performance differs with infection status, e.g. when infected individuals migrate at lower speed or stop-over longer than uninfected individuals. Although such an allochrony has (theoretically) been shown to influence prevalence dynamics (Galsworthy *et al.*, 2011) and may thus play an important role for host-parasite interactions, empirical investigations remain scarce and controversial (DeGroote & Rodewald, 2010).

Haemosporidian parasites, which include the genera *Plasmodium* and *Haemoproteus,* are transmitted via dipteran vectors and commonly occur in avian hosts (Marzal, 2012). These

infections are generally considered relatively benign. However, depending on the particular parasite and/or previous infections, hosts can suffer from weakness and loss of appetite during the acute phase, and rarely even become comatose or die (Valkiūnas, 2005). In most individuals, haemosporidian infections change into a chronic phase (Lapointe, Atkinson & Samuel, 2012), where they may keep elevated leucocyte numbers, while body condition remains unaffected (Figuerola *et al.*, 1999).

Although the diversity and distribution of avian haemosporidians have been studied for decades and described in the context of host migration (Valkiūnas, 1993; Durrant *et al.*, 2008), the influence of avian blood parasites on migrants still remains scarcely investigated (e.g. DeGroote & Rodewald, 2010).

If haemosporidian infections directly or indirectly (e.g. via immune response; Eikenaar & Hegemann, 2016) influence their hosts' energy budgets, we expect infected individuals to stay behind their uninfected conspecifics during migratory flights. Consequently, we predict infected birds to be delayed and prevalence to increase over the passage period – particularly at a stopover site directly following the energy-demanding journey over the Sahara desert and the Mediterranean Sea (Figure 1). Therefore, we linked the timing of passage to haemosporidian infection in several long-distance migratory passerines. Moreover, as a step towards identifying the mechanisms behind infection-related delays, we also included sets of individual, energetic, haematological and biometric variables that might be related to infections in the analysis.

Materials and Methods

Birds were caught on the island of Ventotene (40°48'N, 13°26'E; Spina, 1993) from 17 April to 13 May 2000. We sampled 454 individuals corresponding to 23.5% of the total number of

individuals captured from the four species – pied flycatcher (*Ficedula hypoleuca,* Pallas 1764; n=112), barn swallow (*Hirundo rustica,* Linnaeus 1758; n=103), common redstart (*Phoenicurus phoenicurus,* Linnaeus 1758; n=68) and whinchat (*Saxicola rubetra,* Linnaeus 1758, n=171). Direct observations and near-absence of recaptures indicate that birds were caught within a few hours after landing on Ventotene.

The individual infection status was determined by optical microscopy: On Giemsa-stained thin blood smears we determined intraerythrocytic haemosporidian parasites to genus level with 500x magnification. We recorded parasites of the genus *Plasmodium* and the closely related genus *Haemoproteus*. No leucocyte-inhabiting parasites were found and the few extracellular parasites (Microfilaria, n=1; *Trypanosoma*, n=3) were neglected. Moreover, we counted leucocytes to calculate heterophile-lymphocyte ratio and the sum of all leucocyte types (hereafter called leucocyte sum). Haematocrit was determined by centrifugation in capillaries.

In the statistical analyses, explanatory variables were z-scaled within each species (see Appendices S1 & S2). We related infection status to arrival day in a GLMM (function `glmer`, R package `lme4`, Bates (2015)), using host species as random factor to allow for species-specific intercepts and slopes. We applied Bayesian simulation techniques to compute posterior distributions of the resulting model parameters (function `sim`, R package `arm`). We also tested sets of alternative variables that may explain, or change with, infection status: species, age, daytime of arrival, length of the 8th primary feather, muscle score, fat score, body mass/condition, haematocrit, heterophile-lymphocyte ratio and leucocyte sum (for details see Table S1 in Appendix 1 and Appendix S3 a-e). As leucocyte sum was the only variable significantly related to infection status, we finally fitted a linear regression model to leucocyte sum with infection status and arrival day as explanatory variables for comparing

leucocyte counts independent of arrival day. All analyses were performed in R (R Core Team, 2014).

Results

We detected infections with *Haemoproteus* (n=75), *Plasmodium* (n=24) or both parasite genera (n=33) in 29% of the individuals – with substantial differences between the four host species: While the prevalence of haemosporidian parasites was 20.5% in pied flycatchers, 10.3% in common redstarts and 17.5% in barn swallows, it was as high as 49.1% in whinchats (see Figure 1.1 in ESM1).

The total haemosporidian prevalence increased considerably from 15.7% [95% credible interval (CrI) 10.9/22.2] at the beginning to 31.1% [11.7/60.6] towards the end of the main passage period (Figure 2; GLMM: Intercept=-1.26 [-2.07/-0.46]; slope=0.22 [0.02/0.41]; $p<0.1$). Besides the average prevalence, also its change over time differed among host species: in pied flycatchers from 14.4% [4.6/36.7] to 31.6% [5.1/79.5]; in barn swallows from 12.1% [3.6/34.2] to 28.6% [4.2/78.9]; in common redstarts from 7.8% [2.0/24.9] to 19.8% [2.5/69.1]; in whinchats from 39.5% [16.5/68.8] to 55.4% [14.9/89.9]. These increases were statistically significant for three species (CrIs of slopes: pied flycatcher 0.02/0.41, barn swallow 0.03/0.42, common redstart 0.05/0.44) and marginally non-significant for whinchats (-0.03/0.36).

While none of the other individual, energetic, haematological or biometric variables was related to infection status (GLMMs see ESM3), the leucocyte sum was significantly higher in infected birds compared to uninfected individuals (Figure 2; $F_{2,299}=7.38$, $p<0.001$).

Discussion

We found the prevalence of haemosporidian parasites in migratory passerines to almost double within one month of the main passage period. The haemosporidian prevalences we found lay within the ranges known for these host species (Bensch, Hellgren & Pérez-Tris, 2009). Interestingly, we found an elevated number of leucocytes in the blood of individuals infected with haemosporidian parasites. But apart from this, there were no further differences compared to uninfected individuals, neither in their individual traits, energetic condition and haematological parameters nor in their biometry.

Our findings support the hypothesised migratory allochrony and shows that infection-related delays are detectable – at least after barrier-crossing. As the local prevalence increased within short time and in the midst of the migration period (no change in individual life history state), it is unlikely that within-individual processes caused the observed pattern (Marzal *et al.*, 2016). The increase in prevalence over time was statistically significant in three out of four study species. However, it was marginally non-significant in the whinchat, which already at the beginning of the passage period showed higher prevalence than the other three species at the end. So either, the increase was just slighter, because it already started from a higher prevalence level and is thus difficult to establish. Or varying pathogenicity of different parasites leads to differing influence on host migration patterns (Atkinson *et al.*, 2001). Consequently, these two alternative explanations for the non-significant result for the whinchat may both explain the heterogeneity within our results as well as the results of earlier studies contrasting to ours: For instance, the passage dates of Garden warblers (*Sylvia borin*) were only related to intestinal parasite, but not to blood parasite infections (López *et al.*, 2013) and, while Yellow-rumped warblers (*Setophaga*

coronata) infected with blood parasites were delayed en route in spring, no such differences were found in other species (DeGroote & Rodewald, 2010).

Several mechanisms could explain temporal patterns in prevalence on migration. One candidate mechanism is that resources from endurance exercise are diverted to immune response (Hegemann *et al.*, 2012). Consequently, migrants would need more or longer intermediate fuelling stops or higher fuel reserves for a given distance – both of which would increase total migration duration. Indeed, the elevated leucocyte numbers in haemosporidian-infected birds indicated an up-and-running immune system (Figuerola *et al.*, 1999). We acknowledge that leucocyte counts are a rather coarse measure of immune response and future studies should target combinations of more direct and pathogen-specific measures such as PCRs and antibody assays (Jarvi, Schultz & Atkinson, 2002).

Surprisingly, we found none of the other individual, energetic, haematological or biometric traits to be related to infection status (GLMMs see ESM3). Therefore, the increase in prevalence over time did not result from age- or sex-specific differences in prevalences and migration timing (Hasselquist, 2007).

Infected and uninfected birds did not significantly differ in physical condition, thus infection-related delays seemed not to be caused by anaemia (represented by haematocrit; Fair, Whitaker & Pearson, 2007) or lower body condition (body mass, muscle and fat score; Santiago-Alarcon *et al.*, 2013). Thus, if infections affect energy expenditure during migration these are either compensated (i.e. anticipatory energy accumulation) or go undetected when they lay within the accuracy limits of body condition measures.

Also feather length as proxy for structural body size was unrelated to infection status. Structural size often varies with geographic breeding origin (Rubolini, Spina & Saino, 2005; Hahn *et al.*, 2016). As feather length was not related to infection status and passage date,

the seasonal increase in prevalence on Ventotene cannot be explained exclusively by varying (non-) breeding origins of the passaging birds. Yet, this assumption has not been tested explicitly and earlier studies only provide indirect support: On several Mediterranean islands, including the island of Ventotene, species spending the nonbreeding season at more northern and breeding at more southern latitudes were passing through earlier, compared to those wintering further south and breeding more north (Rubolini *et al.*, 2005). Generally, both occurrence and prevalence of haemosporidian parasites were found to differ among habitats and along latitudinal gradients (Sehgal, 2015). However, it remains to be demonstrated whether wintering area also relates to parasite infection and passage time for populations within species, e.g. by combining parasite screenings with individual tracking.

Under a combination of preconditions, we cannot exclude that also migratory culling – i.e. differential mortality of infected and uninfected individuals on migration – can shape prevalence patterns: If existing, the energetic costs of infection add-up to the energetic costs of migratory flight. If additionally early migration is energetically more costly compared to late migration and early migrants cannot compensate these costs with their commonly assumed better condition (Kokko, 1999), differential mortality could play a role – especially during/after the exhaustive crossing of large barriers. In such a case, migratory culling would bias prevalence more in the beginning and less towards the end of the passage season and differential migration could contribute to the observed prevalence patterns.

In conclusion, infected birds passed Ventotene on average 2.5 days later (1.7d in pied flycatchers, -0.8d in barn swallows, 4.6d in common redstarts and 1.4d in whinchats) than uninfected individuals. Depending on incubation time of haemosporidian parasites in vectors as well as duration of the entire migration and the various stopover periods, even

such small differences in timing could be biologically relevant, as they may reduce the probability of parasite transmission. Moreover, migrants often converge in high densities on stopover sites, making them to transmission hotspots (Hoye, Fouchier & Klaassen, 2012). So irrespective of the mechanistic pathway behind infection-related delays, such migratory allochrony may reduce transmission compared to a synchronously migrating population (Bauer, Lisovski & Hahn, 2016) – and thus, host migration strategy may act as one amongst a multitude of key factors affecting prevalence of populations year-round.

Acknowledgments

This study was funded by the Swiss National Science Foundation with the project 31003A_160265 granted to SB and SH. The article is the publication number 61 of the ''Progetto Piccole Isole'' of ISPRA. We thank the Swiss Tropical and Public Health Institute for providing infrastructure, Susanne Jenni-Eiermann for organizing the lab work and Fränzi Korner-Nievergelt for statistical feedback.

Author contributions

LJ, SBM and FS initiated the study; SBM and FS carried out the field work; SBM screened blood smears; TE, SB and SH designed and performed the analysis, TE drafted the manuscript. All authors revised the manuscript, agreed on the final version and declare no conflict of interest.

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Figures

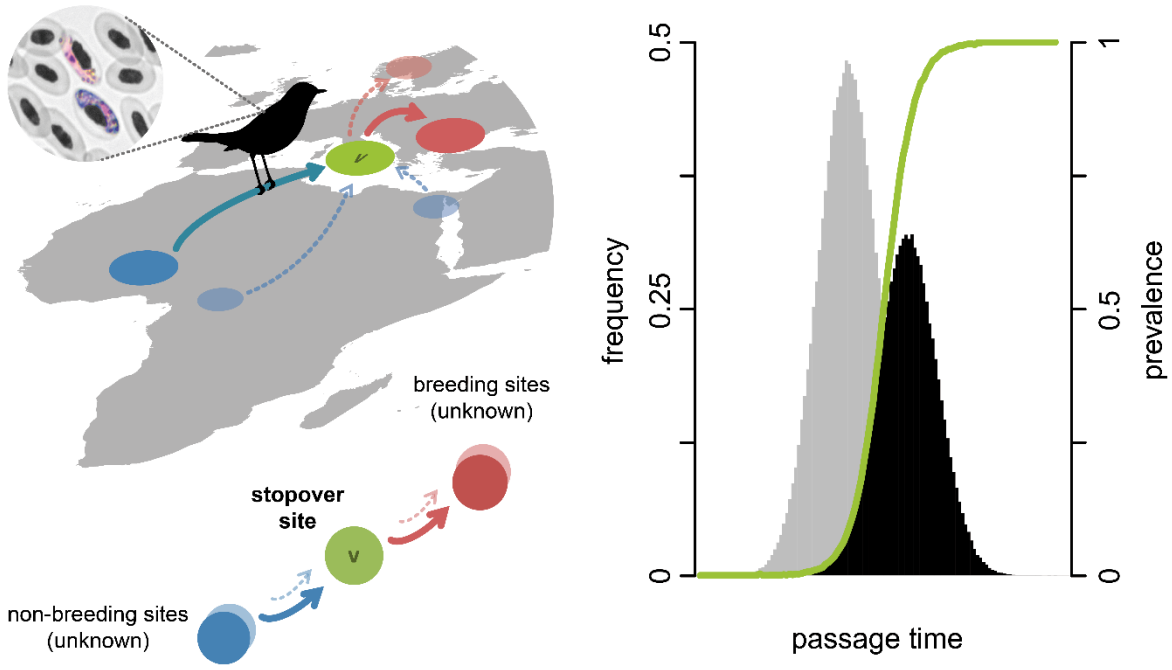

Figure 1: Illustration of pre-breeding migration from sub-Saharan non-breeding to European breeding grounds via a stopover on the island of Ventotene (v) – the presumed first stop after crossing the Sahara Desert and the Mediterranean Sea. If infected (black bars) and uninfected (grey bars) individuals differ in migration timing such that infected individuals lag behind uninfected, we expect increasing prevalence (green line) over time.

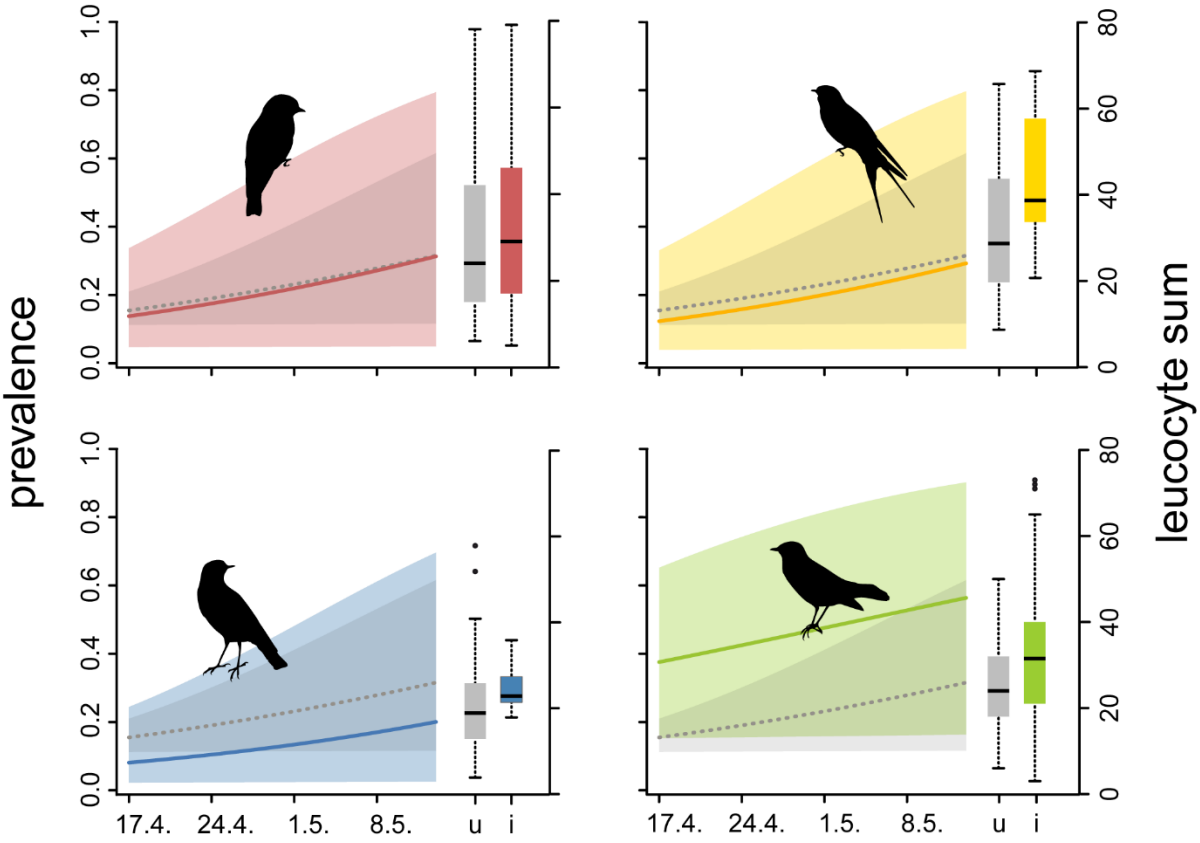

Figure 2: Haemosporidian prevalence (solid line; CrIs as semi-transparent areas) in four species passaging on Ventotene from 17 April to 13 May (from top-left to bottom-right: red = pied flycatcher, yellow = barn swallow, blue = common redstart, green = whinchat; overall model in grey in each subplot). Leucocyte counts in infected (coloured boxes) and uninfected individuals (grey boxes).