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# Evolution of seasonal transmission patterns in avian blood-borne parasites ☆

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ABSTRACT

In temperate regions, many vector-borne parasites maximise their transmission prospects by adjusting reproduction to seasonal cycles of host susceptibility and vector availability. Nevertheless, in these regions there are areas where environmental conditions are favourable throughout the year, so that parasites could benefit from a year-round transmission strategy. We analysed how different transmission strategies (strict summer transmission, extended summer transmission – including spring and autumn, and year round transmission) have evolved among the different genetic lineages of *Haemoproteus parabelopolskyi*, an avian blood-borne parasite shared by three sibling species of passerine hosts. Our results indicate that the ancestral state of this clade of parasites had a strict summer transmission with the blackcap (*Sylvia atricapilla*) as the host. Other transmission strategies and switches to the other host species (*Sylvia abyssinica* and *Sylvia borin*) evolved recently, several times, independently. This suggests that, although year-round transmission is ecologically successful at present, seasonal transmission may have become more stable over evolutionary time. Switches from strict summer to an extended or year-round transmission strategy could have ecological consequences, if they promote the spread of parasites into more distant regions, transported by the migrating bird hosts. Therefore, a deeper knowledge of how different parasite transmission strategies are structured among birds in temperate areas is essential for understanding how disease emergence risks may develop in the future.

## 1. Introduction

The different life histories of parasitic organisms are shaped by natural selection to overcome hosts' defences without causing excessive harm, at least until transmission to other potential hosts has been completed (Frank, 1996). It is well known that host-parasite dynamics depend on environmental conditions (Blanford et al., 2003; Lafferty and Holt, 2003; Mitchell et al., 2005; Wolinska and King, 2009). If parasites are subjected to changes in transmission success driven by environmental seasonal variations, they also must adapt their life cycles to reduce harm to their hosts during periods of no transmission. In temperate regions, where marked environmental changes among seasons regulate life cycles of most organisms, a way of maximising parasite exploitation strategies is to adjust parasite replication to the cycles of host susceptibility and/or vector availability (Altizer et al., 2006). Thus, many parasites spend part of the year as dormant forms and then reappear when transmission conditions are favourable. Typically, transmission peaks during or shortly after periods of host reproduction (generally speaking, spring-summer), as this coincides with an abundance of vectors and susceptible hosts that include immunologically naïve juveniles and adults whose immunity is constrained by reproductive physiological investment (Dowell, 2001; Altizer et al., 2006). Accordingly, parasites display seasonal cycles of covert and overt presence where parasite replication corresponds to seasonal constraints (Sorrell et al., 2009).

Given their key role as drivers of parasite replication and spread, understanding how different seasonal patterns of transmission arise among closely-related parasites could help to predict and prevent current and future disease risks (Morgan et al., 2012). The avian blood-borne parasite, *Haemoproteus parabelopolskyi* (Valkiūnas et al., 2007), is a useful model to study the evolution of different seasonal parasite transmission strategies. Within this vector-borne parasite morphospecies, several closely related genetic lineages can be identified by distinct cytochrome *b* (cyt

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☆ *Note:* New nucleotide sequence data reported in this paper are available in the GenBank™ database under accession numbers JN164702 and JN164703.

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*b*) DNA haplotypes. Lineages behave in turn as proper biological species (as shown by matching phylogenies of mitochondrial (mt)DNA and nuclear (n)DNA markers showing no trace of recombination among lineages; Bensch et al., 2004; Outlaw and Ricklefs, 2014). The *H. parabelopolskyi* lineages have coevolved with a clade of three sibling passerine species of the genus *Sylvia* (Pérez-Tris et al., 2007): (i) the African hill babbler, *Sylvia abyssinica,* which is an Afrotropical resident species, (ii) the garden warbler, *Sylvia borin,* which is a Palaearctic long-distance migrant overwintering in the Afrotropics, and (iii) the blackcap, *Sylvia atricapilla,* which is a Palaearctic species with both resident and migratory populations whose wintering range expands from the south of the Palaearctic region to the Afrotropics. No *H. parabelopolskyi* lineage has ever been found in other bird species outside this clade, and each lineage typically infects only one of the three host species of the clade, with the exception of H-SYAT01 and H-PABY06 lineages, both of which infect both the blackcap and the African hill babbler (Pérez-Tris et al., 2007; Santiago-Alarcón et al., 2011).

Due to the distribution of their three host species, *H. parabelopolskyi* lineages must survive in regions with dissimilar seasonal variations. Therefore, the different lineages have contrasting strategies of seasonal transmission, which allow us to study the pattern of evolutionary transitions between these strategies among otherwise closely related parasites. Most lineages infecting blackcaps and garden warblers seem to be transmitted only in the Palaearctic breeding grounds of their hosts; during winter parasites disappear from the circulating blood and appear only as dormant forms inside their hosts' internal organs. Then parasites relapse into the bloodstream in spring to start a new transmission episode (Pérez-Tris and Bensch, 2005a; Hellgren et al., 2007). Other lineages instead display a continuous presence in the host's bloodstream, enabling transmission throughout the year. This strategy can be found in two different environmental contexts: first, parasites that infect the tropical African hill babbler are not affected by the constraints that winter imposes on transmission in temperate regions. Second, this strategy is also found in some of the parasites that infect the blackcaps that spend winter in the Mediterranean region (either resident or short-distance migrant birds), where climatic conditions are mild enough to allow the activity of insect vectors during winter (Pérez-Tris and Bensch, 2005a; Hellgren et al., 2007).

We set out to analyse how different seasonal patterns of transmission arise among closely related parasites by assessing the pattern of evolution of different seasonal transmission strategies among *H. parabelopolskyi* lineages. To do so, and given that our knowledge of the haemosporidian transmission strategies in temperate regions is still limited to comparisons of parasite prevalences between breeding and mid-winter periods (Pérez-Tris and Bensch, 2005a), our study also reassessed the seasonal transmission patterns of some less well-known lineages by exploring how parasite prevalences progressively change on a monthly basis from the end of one breeding season to the onset of the next. We also looked for seasonal differences in prevalence among parasites, which could offer insight to the relative advantages of one transmission strategy over another. It should be noted that parasite transmission depends on a complex network of factors other than prevalence, such as parasitaemia, vector availability and abundance, and other ecological factors (Cornet et al., 2014); but prevalence can still be considered a good correlate of parasite transmission in haemosporidians (Smith et al., 1993; Beier et al., 1999; Wood et al., 2007; Cornet et al., 2014). All in all, by analysing the evolution of seasonal transmission strategies within phylogenetically restricted clades of parasites and hosts, our study will shed light on the processes promoting such diversity, improving our understanding of putative mechanisms of parasite switching among host species and geographic regions.

## 2. Materials and methods

### 2.1. Field sampling of blackcaps during the winter season

To elucidate how the patterns of summer and year-round transmission of the different lineages of *H. parabelopolskyi* infecting blackcaps vary throughout the wintering season, we performed an extensive parasite screening of wintering blackcaps. The blackcaps were captured in the Campo de Gibraltar region (southern Spain; 36° 01′ N, 5° 36’ W) on a monthly basis, from September 2006 to March 2007, spanning the period during which migratory birds are present in the area in close coexistence with sedentary blackcaps (Cramp, 1992; our personal observations). According to the local climatic conditions, we divided the sampling into three seasons: autumn (September to November), winter (December and January) and spring (February and March).

Shortly after capture, blackcaps were individually identified with a metal ring and sexed and aged by plumage characteristics (Svensson, 1992), distinguishing between juveniles (first-winter birds) and adults (older birds of unknown precise age). The following measures were taken: tarsus length, length of the eighth primary (feather number starting from the body to the wing tip), tail length, and distance between the wing tip and the tip of the first and the ninth primaries. These measures were used to assess the migratory phenotype of blackcaps (migratory or sedentary) using a discriminant function that correctly classifies >97% of birds (Pérez-Tris et al., 1999; De la Hera et al., 2007, 2012). A blood sample (10–20 μl) was collected from the jugular vein, preserved in absolute ethanol and refrigerated until analysed. All birds were released after sampling. Bird sampling was performed in compliance with Spanish environmental regulations and with the authorisations issued by the Spanish Ministry of Environment and the Government of the Autonomous Region of Andalucía, Spain.

### 2.2. Molecular analyses

Total DNA was extracted from blood samples following a standard ammonium acetate protocol. DNA was diluted to a working concentration of 25 ng/μl. Parasite presence was assessed through the nested PCR protocol developed by Waldenström et al. (2004) to detect infections of avian *Plasmodium* and *Haemoproteus,* in which a 479 bp fragment of the parasite's cyt *b* gene was amplified. PCR results were verified in 2% agarose gels, stained with ethidium bromide, and assessed under UV light to look for bands of appropriate size. Negative samples were rerun to look for false negatives. Both a positive (DNA template from an infected bird) and negative (distilled water instead of template DNA) PCR control were included in every PCR batch to test for quality and contamination. No negative control yielded a positive result. All samples were previously tested using a standard bird sexing protocol (Griffiths et al., 1998) to test for sample quality and PCR suitability.

Positive samples were sequenced on an ABI Prism 3730 capillary robot (Applied Biosystems, UK) using the primer HaemF (Waldenström et al., 2004). Sequences were edited manually using BioEdit 7.0.5.3 (Hall, 1999) and unique cyt *b* gene haplotypes were defined by a sequence divergence of at least one base in the amplified fragment. Mixed infections were detected by the presence of double peaks on the electropherograms (Pérez-Tris and Bensch, 2005b) and the identity of the parasites involved was assessed comparing the double peak patterns with the previously known parasite lineages infecting blackcaps. Parasite DNA haplotypes, which were not previously described, were confirmed by repeating the PCR and then sequencing from both ends with primers HaemF and HaemR2. New sequences were given a name according to MalAvi nomenclature (Bensch et al., 2009) and registered in GenBank.