Early and Late Migrating Avian Individuals Differ in Constitutive Immune Function and Blood Parasite Infections – But Patterns Depend on the Migratory Strategy

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Billions of birds migrate every year. To conduct a successful migration, birds undergo a multitude of physiological adaptions. One such adaptation includes adjustments of immune function, however, little is known about intraspecies (between-individual) and interspecies (between-species) variation in immune modulations during migration. Here, we explore if early and late migrating individuals differ in their immune function, and if such patterns differ among species with short- vs. long-distance migration strategies. We quantified three parameters of baseline (constitutive) innate immune function and one parameter of baseline (constitutive) acquired immune function in 417 individuals of 10 species caught during autumn migration at Falsterbo (Sweden). Early and late migrating individuals differed in lysis and total immunoglobulins (IgY), but the patterns show different directions in long-distance migrants (LDMs) (wintering in Africa) as compared to short-distance migrants (SDMs) (wintering within Europe). Specifically, early migrating LDMs had lower lysis but higher immunoglobulin levels than late migrating individuals. In short distance migrants, there was no difference in lysis between early and late migrating individuals, but immunoglobulin levels were higher in late migrating individuals. We found no correlation between timing of migration and haptoglobin, but LDMs had lower levels of haptoglobin than SDMs. We also found that the prevalence of haemosporidian blood parasite infections decreased in LDMs, but increased in SDMs, as the autumn progressed. Taken together, our study suggests that the investment into immune function depends on the migratory strategy (short- vs. long-distance migrants), and that early and late migrating individuals of a migration strategy might invest differently in baseline immune function, potentially driven by differences in the trade-offs with timing and speed of migration. Our study highlights the potential adaptations of immune function that could help explain trade-offs with other physiological systems, and behavioural responses during migration.

Keywords: optimal migration theory, ecophysiology, avian migration, stopover ecology, eco-immunology, migratory strategy, avian malaria

INTRODUCTION

Each year billions of birds migrate from breeding to non-breeding grounds. The timing of migration, as well as the distance covered between the breeding and the wintering grounds, differs greatly both among and within species. Species migrating to winter in sub-Saharan Africa typically migrate in early autumn and are often referred to as long-distance migrants (LDMs) (Berthold, 1993; Newton, 2008). In contrast, species migrating from Northern Europe to winter in western or southern Europe are often referred to as short-distance (or medium-distance) migrants and typically migrate later during the autumn season (Berthold, 2001; Newton, 2008). LDMs are hypothesised to be time-minimisers, selected to arrive at the wintering grounds as early as possible (Alerstam and Lindström, 1990; Hedenström and Alerstam, 1997; Alerstam et al., 2003). On the other hand, short-distance migrants (SDMs) are more likely to be energy-minimisers (Alerstam and Lindström, 1990; Hedenström and Alerstam, 1997). During migration, birds need to adjust their physiology to a multitude of challenges, including but not limited to environmental changes and sustained periods of extreme physical exertion (Piersma and van Gils, 2011). During the last decades, studies on the physiological adjustments required during migration have focused on energetic aspects (and hormonal adjustments) (Blem, 1980; Alerstam and Lindström, 1990; Gwinner, 1990; Lindström and Alerstam, 1992; Lindström, 2003; Guglielmo, 2010; Piersma and van Gils, 2011). However, recent studies indicate that there are also a number of other physiological systems adjusted during migration (Piersma and van Gils, 2011; Cooper-Mullin and McWilliams, 2016; Hegemann et al., 2019; McWilliams et al., 2021). In particular, some studies have highlighted that migration is connected with changes in the immune system that can influence migratory behaviour and processes. For example, both constitutive immune function and responsiveness to immune challenges can be reduced during the migratory season (Owen and Moore, 2006, 2008a,b; Buehler et al., 2008b, 2010b; Eikenaar and Hegemann, 2016). Also trade-offs between immune function and other physiological systems like oxidative balance may occur during migration (Eikenaar et al., 2018) and stopovers may not only be required for refuelling, but also for recovery of immune function (Owen and Moore, 2008b; Buehler et al., 2010b; Eikenaar et al., 2020a,b). Furthermore, levels of constitutive immune function relate to stopover duration (Hegemann et al., 2018b) and to the length of migration delays if individuals need to mount an immune response during the migratory season (Hegemann et al., 2018a). However, many questions regarding these physiological adaptations still remain open (Schmaljohann and Eikenaar, 2017; Hegemann et al., 2019).

The immune system protects the body from pathogens and other non-self particles and is therefore important for survival (Roitt et al., 1998), but at the same time, it incurs costs in terms of production, maintenance and activation (Klasing, 2004; Hasselquist and Nilsson, 2012). Several studies found evidence for downregulation of (parts of) constitutive immune function during migration (Owen and Moore, 2006; Buehler et al., 2010b; Eikenaar and Hegemann, 2016), presumably

resulting from resource or risk trade-offs (Owen and Moore, 2008b; Buehler et al., 2010b; Eikenaar et al., 2020a). However, information whether there are general differences between species with different migratory strategies [e.g., long-distance vs. short-distance migrants (SDMs)] is currently lacking. Migratory strategies may also lead to different trade-offs with the demands for migration itself, resulting in differential investment in immune function (Hasselquist, 2007; Buehler and Piersma, 2008; Buehler et al., 2010a). In particular, LDMs have been hypothesised to have a higher susceptibility to infectious diseases, because the high physiological demands of long-distance migration may lead to reduced immune function (Klaassen et al., 2012). Furthermore, both the timing and the destination of migration may influence the risk of exposure to pathogens encountered by the migrants (Altizer et al., 2011; Hall et al., 2016). For example, many infectious diseases as well as vectors of pathogens show seasonal patterns and hence early migrating individuals and species may be exposed to other disease risks than late migrating species or individuals (Altizer et al., 2006; van Dijk et al., 2014), and tropical areas are supposed to harbour more pathogens than temperate regions (Altizer et al., 2011; Westerdahl et al., 2014). Finally, within species there is considerable variation in timing of migration, which may result in individual differences in trade-offs between immune function and migration. Yet, comprehensive data on seasonal patterns of immune function among long- and short-distance migrants, as well as early and late migrating individuals within a species, are lacking. Such knowledge would also help to understand disease transmission patterns and to identify the time windows with the highest risk of spreading of disease (Owen et al., 2021).

A recent meta-analysis of published papers on infection status and infection intensity in migratory hosts found that across animal taxa, parasites have a negative impact on migration performance and that migratory performance decreased with increasing infection intensity (Risely et al., 2018). Hence, infected birds migrate more slowly and may therefore lag behind conspecifics. Yet, data on temporal segregation of infected birds across a migratory season are still rare. An analysis of four passerine species on spring migration revealed that late migrating individuals had a much higher prevalence of haemosporidian blood parasites [one of the most common chronic infections in birds (Valkiūnas, 2004)] than early migrating individuals (Emmenegger et al., 2018). In Yellow-rumped Warblers *Dendroica coronate* during spring migration, both the probability and the intensity of blood parasite infections were higher for later migrating birds (DeGroote and Rodewald, 2010). Both studies suggest that blood parasite infections may hamper spring migration (see also Garvin et al., 2006) or that reduced feather growth during moult in infected individuals may delay the onset of spring migration (see Marzal et al., 2013). Indeed, during autumn migration, SDMs infected with avian haemosporidians had three times as long stopover periods as uninfected birds, though there was no difference in stopover duration between infected and uninfected LDMs (Hegemann et al., 2018b). However, that study did not investigate effects of seasonal timing. To our knowledge, the only study investigating seasonal patterns of blood parasite infections in

autumn migrating birds found that late migrating European Robins *Erithacus rubecula* tended to have higher prevalence of avian malaria than early migrating individuals (Agh et al., 2019). Hence, comprehensive data on temporal differences between infected and non-infected individuals during autumn migration are still missing.

In this study, we investigated if LDMs and SDMs differ in baseline (constitutive) immune function and if, within either group, early-migrating individuals differ from late migrating individuals. We sampled 417 individual birds of 10 species during autumn migration stopover at Falsterbo (Sweden). Our aims were to quantify (1) if early migrating individuals (within a species) have different immune function or different haemosporidian prevalence compared to late migrating individuals, and (2) if these patterns differ between LDMs and SDMs. We hypothesised that due to stronger selection for time-minimisation, late LDMs would have lower baseline immune function compared to early migrating individuals as they need to invest more resources in fast migration. In contrast, we did not expect any differences in immune function between early and late migrants among SDMs as all of them were relatively close to their final destination and therefore not time-stressed. Regarding blood parasite infections, following earlier work during spring migration, we expected late individuals to have higher prevalence of blood parasites than early migrating individuals independent of the migration strategy.

MATERIALS AND METHODS

We sampled passerine species during the 2014 autumn migration season at the Falsterbo Peninsula, a strategic stopover site in Southwest Sweden (55.383°N, 12.816°E). Birds were caught as part of a standardised ringing project in the lighthouse garden assumed to be migrants during stopover (Karlsson and Bentz, 2004; Hegemann et al., 2018a,b). We sampled birds between 26 August and 11 November. Birds were caught between sunrise and 12:45 h CET and mistnets (maximum of 21 nets of 9 m each; 16 mm mash size, see Karlsson and Bentz, 2004) were checked every half hour and birds were brought for further handling into the ringing hut. Birds were aged based on plumage characteristics (Svensson, 1992) and we included only hatch-year birds (which constitute about 90% of all ringed birds at Falsterbo; Falsterbo Bird Observatory, unpublished data) in our study. All species of which less than 10 individuals were sampled were excluded, resulting in samples from 417 individuals of 10 species. Six of those species (Blackbird *Turdus merula*, $n = 15$, Blue Tit *Cyanistes caeruleus*, $n = 38$, Chaffinch *Fringilla coelebs*, $n = 24$, Dunnock *Prunella modularis*, $n = 63$, Robin *E. rubecula*, $n = 98$, and Song Thrush *Turdus philomelos*, $n = 62$) are SDMs that winter in Western and Southern Europe, and four species (Common Redstart *Phoenicurus phoenicurus*, $n = 33$, Garden Warbler *Sylvia borin*, $n = 20$, Tree Pipit *Anthus trivialis*, $n = 27$, Willow Warbler *Phylloscopus trochilus*, $n = 37$) are LDMs that winter in sub-Saharan Africa.

We collected blood samples ($\sim$100 μl; always less than 10% of an individual's total blood volume) by puncturing the brachial vein with a sterile 27G needle and transferring blood *via* heparinised capillary tubes (Hirschmann Laborgeräte GmBH, Germany) into Eppendorf tubes. Blood samples were kept on ice in an Eppendorf tube until we returned to the lab the same day, and then centrifuged for 10 min at 7000 rpm to separate plasma from red blood cells. Samples were stored in Eppendorf tubes at $-50°$C until subsequent laboratory analysis.

Immune Assays

We used two assays that focus on the innate immune system and one assay that focus on the acquired immune system (Hegemann et al., 2018a,b). The innate immune system constitutes the important first line of defence (Janeway et al., 2005). Levels of constitutive innate immune function relate to pathogen pressure (Horrocks et al., 2012, 2015) and show consistencies over longer time scales (Hegemann et al., 2012), but also experience modulations during migration (Eikenaar et al., 2020a,b). Specifically, we used a haemolysis-haemagglutination assay to quantify titres of complement-like lytic enzymes (measured as lysis titres) and non-specific natural antibodies (measured as natural antibody titres) and followed the method described by Matson et al. (2005). Scanned images of individual samples serially diluted in Rabbit red blood cells (Alsevers, S.BC-0009, Envigo, United Kingdom) were randomised among all plates and scored blindly to sample ID (by AH). If scores differed by >1, then a third score was taken and the median was used as a final value. We used a commercially available colorimetric assay kit (Tp801; Tridelta Development Ltd.) to quantify haptoglobin concentrations in plasma samples (Matson et al., 2012). We followed the manual method as described in the manufacturer manual with a few minor modifications. We measured absorbance at three wavelengths (405, 450, and 630 nm) prior to the addition of the final reagent that initiated the colour change reaction. The 405 and 450 nm pre-scan enabled us to statistically analyse and correct for differences in plasma sample redness, an indication of haemolysis, which can affect the assay (Matson et al., 2012; Wemer et al., 2021). Haptoglobin is an acute phase protein that circulates in the blood and during a pathogenic challenge haptoglobin concentration typically increases following release from the liver. Acquired immune function reflects the investment into immune function over longer time scales and hence is likely related to phenotypic quality (Hasselquist et al., 2001). We used an enzyme-linked immunosorbent assay (ELISA) to quantify the total level of antibodies (IgY) following the methods exactly as outlined in Sköld-Chiriac et al. (2014). Samples were defrosted and refrozen between each assay, but these assays are robust to repeated freeze-thaw cycles (Hegemann et al., 2017).

Molecular Analyses of Blood Parasites

DNA from preserved blood was extracted using standard phenol/chloroform methods (Sambrook et al., 2002) and subsequently diluted to 25 ng μl^{-1} (concentration determined using a nanodrop). To determine infection status with haemosporidian species of the genera *Haemoproteus*, *Plasmodium*, and *Leucocytozoon*, we used a nested PCR amplifying a partial segment of the cytochrome *b* gene and

using both Haem-F/Haem-R2 and Haem-FL/Haem-R2L primer pairs following Hellgren et al. (2004). We subsequently grouped the data by birds having no infection, birds having one infection (*Haemoproteus/Plasmodium* or *Leucocytozoon*), or birds having double infection (*Haemoproteus/Plasmodium* and *Leucocytozoon*). Since we only consider first year birds in our analyses, all infections must have occurred on the breeding grounds and birds cannot yet be infected with lineages that are transmitted on the wintering grounds (see Hellgren et al., 2007).

Statistics

We analysed our data using linear mixed models [function lmer, package lme4 (Bates et al., 2015)] and the program R version 3.6.3 (R Development Core Team, 2020). Even though measurements of baseline immune function are usually not affected by short-term handling stress (Buehler et al., 2008a; but see Zylberberg, 2015), we first checked if handling time and immune parameters were correlated in our dataset, but this was not the case for any parameter (always $F < 2.31$, $p > 0.13$).

To separate within-species effects from between-species effects, we applied the methods described by van de Pol and Wright (2009). We used the immune parameters and blood parasite infection as dependent variable and the two-way-interactions of within-species-date effect and strategy (long-distance vs. short distance migrant), and between-species-date effect and strategy as explanatory variables. Species was included as random intercept effect. The model with haptoglobin included a measurement at 405 nm as covariate to correct for plasma redness as this measurement explained more variation than then 450 nm measurement (Matson et al., 2012; Wemer et al., 2021). Blood parasite infections were grouped into three levels (no infection, single infection, double infection).

We always started with the full model and removed interactions if $p > 0.1$ based on backward elimination and the drop1 function in R. We kept all fixed effects independent of significance. Assumptions of all models were checked on the residuals of the final model. Residuals from the model having agglutination as dependent variable deviated from normality and transformations did not improve the fit. However, since general linear models are robust against deviation from normality (Zuur et al., 2010), we still consider the results to be valid. We extracted and plotted marginal means using the function ggpredict [package ggeffects (Lüdecke, 2018)].
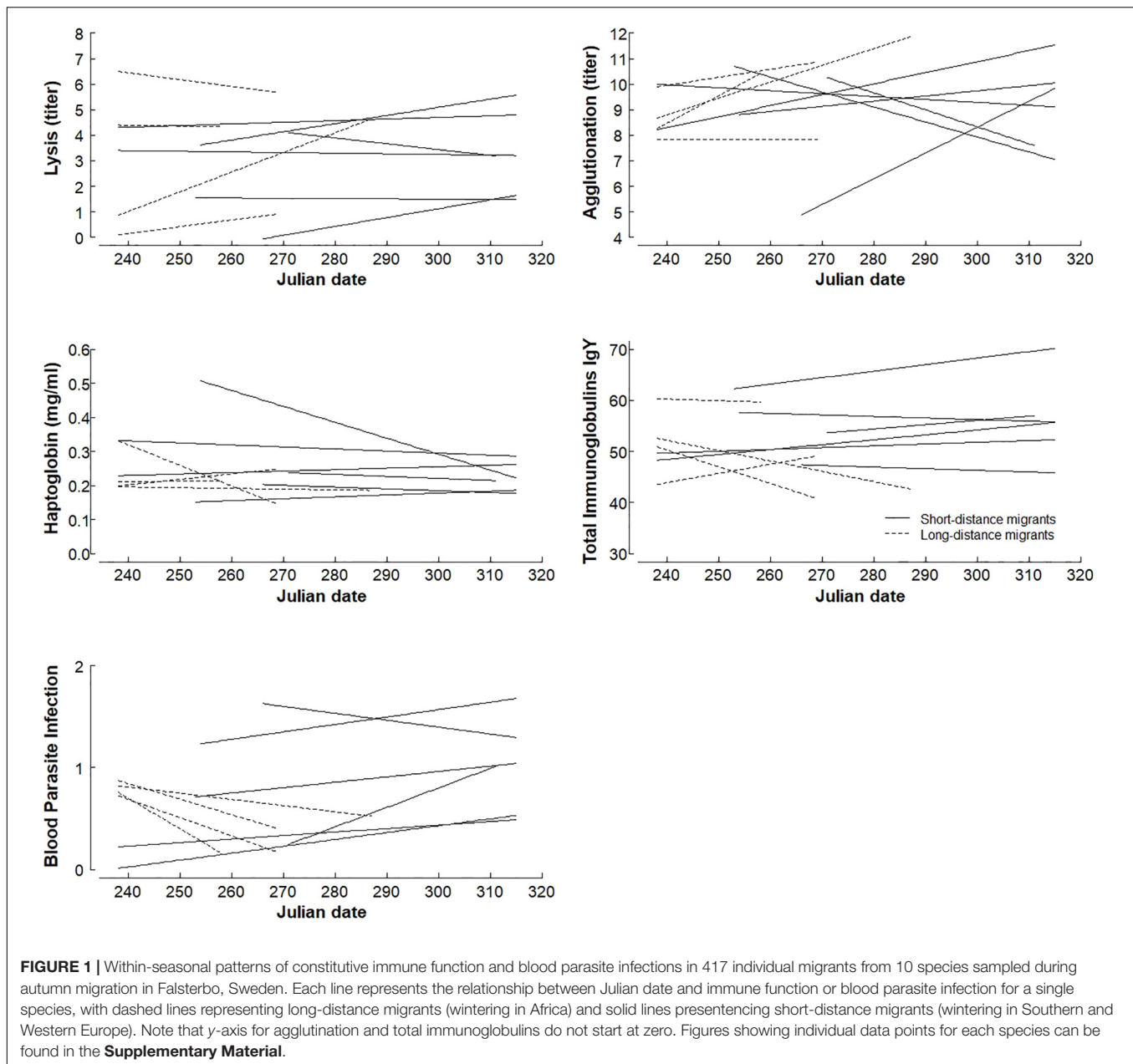
RESULTS

Overall, early migrating individuals of LDMs had lower lysis, higher immunoglobulin levels and a higher prevalence of blood parasite infections than late migrating individuals (**Figures 1**, **2** and **Table 1**). In contrast, among SDMs, early individuals did not differ from late individuals in lysis, but had lower immunoglobulin levels and lower prevalence of blood parasite infections than late migrating individuals (interaction within-species-date-effect × strategy: lysis: $p = 0.062$, immunoglobulins: $p = 0.048$, blood parasite infections: $p = 0.005$, **Figures 1**, **2**, for full statistical results, see **Table 1**). Agglutination and haptoglobin

concentrations did not differ between early and late migrating individuals, and there was no effect of migration strategy (interaction within-species-date-effect × strategy: agglutination: $p = 0.283$, haptoglobin: $p = 0.713$; within-species-date effect: agglutination: $p = 0.404$, haptoglobin: $p = 0.984$, **Figures 1**, **2** and **Table 1**). In general, LDMs had lower haptoglobin concentrations ($p = 0.049$) and a non-significant trend for lower agglutination ($p = 0.087$) than SDMs (**Table 1**). Species-specific figures showing all individual data points can be found in the **Supplementary Figures 1–5**.

DISCUSSION

We show that several parameters of constitutive (baseline) immune function and blood parasite infections differ between early and late migrating individuals, and that these patterns differ between long- and short-distance migrants. Thus, our study suggests that over the autumn migration period immune function is modulated in different ways depending on migration strategy.

Specifically, early migrating LDMs had lower complement activity (lysis) than late migrating individuals, while there was no such difference in SDMs. That early and late individuals of LDMs differ indicates a trade-off with migration timing in these time-minimisers (Klaassen et al., 2012). Individuals migrating early may have to make a trade-off with investment in immune function, in this case complement activity, a parameter that has been linked to survival probabilities (Hegemann et al., 2015; Roast et al., 2020). While early departure from Scandinavia might be beneficial in terms of early crossing of the Sahara and hence early arrival at the wintering grounds (Alerstam and Hedenström, 1998) this may come at the cost of innate immune function which still develops in young birds after independence (Stambaugh et al., 2011; Vermeulen et al., 2017; van Dijk et al., 2020; Aastrup and Hegemann, 2021). The higher immunoglobulin levels in early migrating LDMs compared to late migrating individuals may seem to contradict this hypothesis. However, immunoglobulin levels are thought to reflect phenotypic quality/condition (Hasselquist et al., 2001) and particularly LDMs that will encounter more novel pathogens, may benefit from investing in this part of immune function to be prepared for entering (tropical) wintering quarters with a higher pathogen pressure (Westerdahl et al., 2014; O'Connor et al., 2020). Low immunoglobulin levels among late migrating LDMs may then suggest that these individuals are of lower quality/condition and that they can therefore not initiate migration as early as those with high immunoglobulin levels. For SDMs, immunoglobulin levels instead increased over the autumn migration period. This may reflect that among SDMs high quality/condition individuals are migrating later in the autumn. Alternatively, this might be an effect of that the levels of circulating immunoglobulins are increasing over the autumn in these young birds as they are exposed to more and more pathogens. Such an effect may be particularly visible in SDMs as those birds are on average 2 months older when they migrate as compared to LDMs, because SDMs hatch earlier and migrate later.

FIGURE 1 | Within-seasonal patterns of constitutive immune function and blood parasite infections in 417 individual migrants from 10 species sampled during autumn migration in Falsterbo, Sweden. Each line represents the relationship between Julian date and immune function or blood parasite infection for a single species, with dashed lines representing long-distance migrants (wintering in Africa) and solid lines presentencing short-distance migrants (wintering in Southern and Western Europe). Note that y-axis for agglutination and total immunoglobulins do not start at zero. Figures showing individual data points for each species can be found in the **Supplementary Material**.

Natural antibodies (agglutination) did not differ between early and late migrating individuals, which may suggest that this part of immune function is not involved in trade-offs related to migration. In line with this, several previous studies failed to find any correlation between agglutination and migration related traits (Buehler et al., 2010b; Nebel et al., 2012, 2013; Hegemann et al., 2018a,b; Eikenaar et al., 2020b). These studies as well as our current study instead suggest that other immune parameters (e.g., complement activity, haptoglobin concentration, immunoglobulins, bacteria killing capacity) seem to be more important for trade-offs between immune function and physiological or behavioural adaptations related to migration.

Haptoglobin levels did not differ between early and late migrating individuals. Since haptoglobin is an acute phase protein that increases during infection (Quaye, 2008), similar levels between early and late individuals might not be surprising. Interestingly though, LDMs had on average lower haptoglobin concentrations compared to SDMs. Besides its role for immune function, haptoglobin has antioxidant properties as it can bind haemoglobin, a mechanism potentially important during long endurance flights as haptoglobin levels were negatively correlated with flight duration in European Starlings (*Sturnus vulgaris*) flying in a wind tunnel (Nebel et al., 2012). Since LDMs tend to fly longer distances during each migratory flight compared
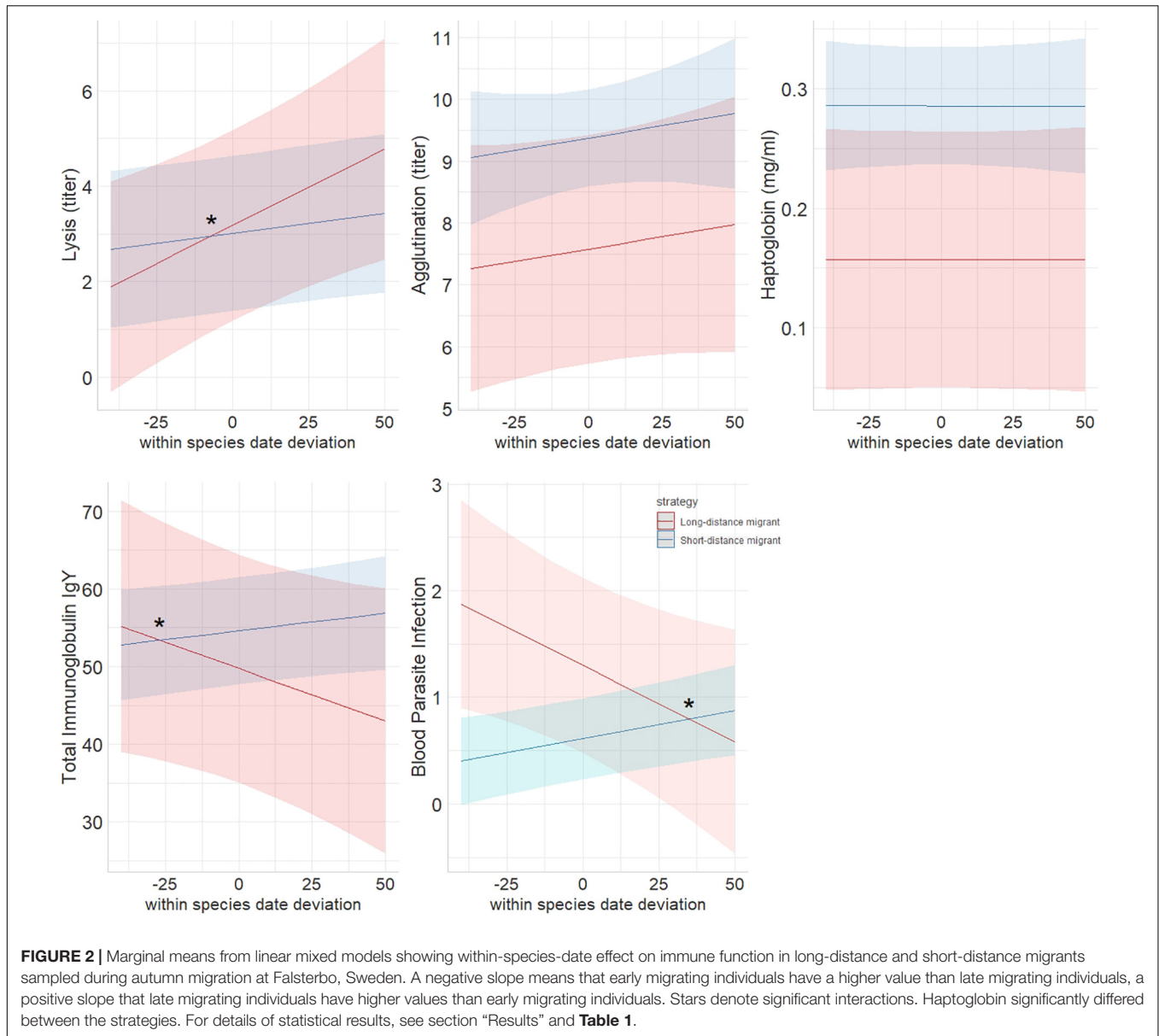
FIGURE 2 | Marginal means from linear mixed models showing within-species-date effect on immune function in long-distance and short-distance migrants sampled during autumn migration at Falsterbo, Sweden. A negative slope means that early migrating individuals have a higher value than late migrating individuals, a positive slope that late migrating individuals have higher values than early migrating individuals. Stars denote significant interactions. Haptoglobin significantly differed between the strategies. For details of statistical results, see section "Results" and **Table 1**.

TABLE 1 | Statistics and coefficients for linear mixed models analysing immune function and blood parasite infections in migrating birds during autumn migration at Falsterbo, Sweden.

	Lysis		**Agglutination**			**Haptoglobin**			**Immunoglobulins**		**Blood parasites prevalence**		
X-variable	**F**	**p**	**Beta**	**F**	**p**	**Beta**	**F**	**p**	**F**	**p**	**Beta**	**F**	**p**
Strategy: between-species-date	5.17	**0.023**		2.19	0.139		0.25	0.620	1.84	0.175		0.13	0.717
Strategy: within-species-date	3.48	*0.062*		1.15	0.238		0.14	0.713	3.91	**0.048**		7.98	**0.005**
Between-species-date			−0.07	3.92	**0.048**		2.01	0.156	0.03	0.855	0.03	3.87	**0.049**
Within-species-date				0.70	0.404		0.00	0.984					
Strategy*			1.80	2.93	*0.087*	0.13	3.86	**0.049**					
Redness	NA	NA		NA	NA	0.59	384.3	**<0.001**	NA	NA		NA	NA

Within- and between-species effects were separated following the methods described by van de Pol and Wright (2009). Strategy: migration strategy (long-distance migrants vs. short-distance migrants). Redness: plasma redness to correct for plasma colour differences; relevant only for haptoglobin. p-Values < 0.05 are bolded, p-values < 0.1 > 0.5 are italics.
*Short-distance migrant as reference.

to SDMs (Schmaljohann, 2019; Packmor et al., 2020), this may explain the difference in haptoglobin between long- and short-distance migrants.

Prevalence of blood parasite infections increased in late migrating SDMs compared to early migrating birds and this is in line with findings in autumn migrating Robins in Hungary (Agh et al., 2019). An increase in blood parasite prevalence over the autumn in SDMs also supports the hypothesis that infected individuals migrate more slowly (Risely et al., 2018) and thus later, for example because of longer stopovers or shorter flight bouts (Hegemann et al., 2018b). Furthermore, infection with blood parasites can reduce feather growth (Marzal et al., 2013) and may delay the onset of migration in infected individuals. In contrast, migrating LDMs' prevalence of blood parasites decreased over the autumn migration period, which is contrary to our prediction and to previous results for LDMs [though during spring migration (Emmenegger et al., 2018)]. This contradicts the hypothesis of infected migrants being slowed down. However, since LDMs have the majority of their migratory flight still ahead of them, we cannot rule out that infected individuals migrate more slowly and hence are lagging behind uninfected individuals and that this pattern may only be visible later in the migratory season. Alternatively, but not mutually exclusive, early departure from the breeding grounds of infected individuals may be an adaptive strategy if they can anticipate that blood parasite infection may slow them down along the route. Another explanation may include selective disappearance of late migrating low-quality individuals infected with blood parasites (Marzal et al., 2016). Specifically, if late migrating long-distance migratory individuals are of poorer quality, as indicated by their lower levels of immunoglobulins (see above), those low-quality individuals may have reduced capacities to fight a haemosporidian infection (Gonzalez et al., 1999) and hence experience higher mortality during infection than early migrating high-quality individuals. This explanation may also resolve the apparent contradiction that early migrating LDMs have both high immunoglobulin levels (indicating high quality) and high prevalence of blood parasites. It seems unlikely that infections had already retracted into organs and were undetectable in the blood (Cosgrove et al., 2008), because prevalence increased over time and was on average much higher in late migrating SDMs. Furthermore, in a local bird community in southern Sweden, several long-distant migratory species showed no trend in parasite prevalence in late summer and early autumn when considering only first-year birds (Huang et al., 2020), and in blue tits *Plasmodium* blood parasites become dormant only from the end of October onwards (Cosgrove et al., 2008). Even though the overall patterns between blood parasite infections and immunoglobulins seem to parallel each other, we found no correlation on the individual level (Hegemann et al., in preparation).

CONCLUSION AND FUTURE DIRECTIONS

Our study adds to the growing literature that other physiological systems than those directly linked to energy turnover are modulated and thus variable during migration, and that this may be particularly true for the immune system (Buehler et al., 2010a; Hegemann et al., 2019) with potentially substantial impact on fitness-related factors (Roast et al., 2020; Dunn et al., 2021). That the different immune parameters did not show any common time patterns over the autumn migration period may highlight the different functions and costs of different immune parameters (Adamo, 2004; Matson et al., 2006; Demas et al., 2011) and that these trade-offs may differ between migration strategies. It also suggests that there is no general down-regulation or up-regulation of immune function, but that different parameters show different patterns within species, as well as between species using different migration strategies (i.e., short- and long-distance migrants). Hence, our results stress the importance of measuring multiple immune parameters in migration studies. The differences between early and late individuals also suggests that trade-offs between immune function and migration speed or other physiological systems might change over time as the migration season progresses. Future studies should aim at getting repeated samples from the same individuals both at the same stopover sites as well as during different stopovers along the migratory route to understand how flexible or fixed different immune strategies are. Satellite tracking of birds as well as radio-tracking in areas covered by a network of receiving stations like the Motus system (Taylor et al., 2017) may offer such possibilities. Understanding such variations in immune function will not only be vital for understanding the ecology and evolution of migration (Hegemann et al., 2019), but may also be important for predicting the interplay between birds and diseases under climate change (Hall, 2021).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors on request.

ETHICS STATEMENT

The animal study was reviewed and approved by the Malmö/Lund Ethical Committee.

AUTHOR CONTRIBUTIONS

AH, J-ÅN, and DH: study design. AH: field work, statistical analyses, and first draft of the manuscript. AH and CB: labwork. AH, CB, DH, and J-ÅN: discussion and interpretation of data. All authors gave substantial input on the manuscript and approved the submitted version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.880426/full#supplementary-material

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