Causes and Consequences of Avian Migration: An Assessment of Ectoparasite Infections and Immune System Evolution

by

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Abstract

Migration is a widespread behavior observed across many mobile organisms, such as mammals, insects, fish, and birds. Migration has evolved independently in multiple ecological contexts, but the consequences of its evolution on species' life history are not well delimited. This dissertation investigates the hypothesis that migration influences the degree of parasitism and the diversity of the Major Histocompatibility Complex, which is a part of the adaptive immune system. In the first data chapter, I examined ectoparasite infections among salvaged museum bird specimens from migratory and resident species collected during the fall and spring migratory seasons. I used Bayesian mixed modeling approaches to test the relationship between migration and seasonal variation in ectoparasite presence among migratory and resident birds that have similar breeding distributions. First, I tested the prediction that ectoparasitism is greater after the breeding period, corresponding to the onset of fall migration, compared to at the end of the migratory periods, as expected under the hypothesis that migration is a strategy to escape increasing parasitism in the breeding range. Contrary to this expectation, I observed a higher incidence of ectoparasite infections upon arrival near the breeding range in the spring than during departure in the fall. This suggests that ectoparasitism does not increase throughout the breeding period and does not facilitate seasonal departure from the breeding ranges. Furthermore, I identified a positive relationship between migration distance and ectoparasite infections in spring, suggesting that a longer migration distance predicts an increase in parasitism. This is consistent with the idea that long-distance migratory species may experience higher parasitism because of the physiological stress of migration and/or because their nonbreeding ranges are at

lower latitudes and are expected to be pathogen-rich compared to nonbreeding ranges at higher latitudes. In my second data chapter, I quantified the genetic diversity of the Major Histocompatibility Complex Class I (MHC-1), which is a genomic region involved in the adaptive immune response that evolves though pathogen-mediated selection. I designed the study to compare MHC-1 diversity among closely related species within four genera that breed in sympatry but have different migration distances. I found similar levels of MHC-1 diversity among all species, which is inconsistent with the expectation that long-distance migration is associated with higher immune diversity owing to exposure to higher pathogen diversity. In summary, the studies in this dissertation these results suggest that migratory birds experience increased ectoparasitism during migration compared to during the breeding season, but that the diversity of the adaptive immune response is similar among birds that migrate different distances. Overall, the findings of this dissertation suggest that it is unlikely that parasitism is a central evolutionary driver of avian migration. Instead, parasitism is a consequential challenge to which migratory birds must adapt.

Chapter 1 Introduction

Seasonal migration, which is an annual round trip movement between disjunct breeding and nonbreeding ranges[1], is a common behavior across mobile organisms such as mammals, insects, fish, and birds. Although there is a broad diversity of migratory phenotypes, migratory taxa exhibit some common traits[2]. Seasonal migrants must be mobile, able to navigate between ranges, and have chronobiological mechanisms to ensure appropriate departure and arrival timing. For migration to evolve as an adaptive strategy, migratory populations must adjust to the trade-offs between survival and fecundity, which are associated with long-distance movements[2]. Migration has evolved multiple times, suggesting a multitude of conditions that can favor its evolution[1]. Empirical studies (see[3]) support several hypotheses on the evolution of migration; however, the contributions of each hypothesis to the evolution of different migratory behaviors are not well studied.

1.1 Migration as a strategy for parasite avoidance

One hypothesis for the evolution of migration describes parasite and pathogen avoidance are central drivers of migration[4–7]. Parasites and pathogens are among the strongest selective forces on hosts because they harm the host and reduce survival[8–10] and reproductive success[10–14]. In response, selection for parasite avoidance has contributed to the evolution of behaviors, such as grooming[15] and nest sanitation[16], which illustrates the degree to which parasitism can drive evolution[15,17,18] and possibly the evolution of migration[19,20].

The migratory escape[6,7] hypothesis proposes that seasonal migration may have evolved as an adaptive strategy that benefits survival and reproductive success by allowing migrants to escape parasitism in increasingly infested habitats. This hypothesis predicts that migratory hosts experience reduced parasitism by evacuating and avoiding seasonally infested habitats and through the culling of highly infected individuals, which reduces parasite prevalence and transmission rates among hosts and may select for less virulent pathogens [6,21].

The best evidence supporting migration as a mechanism that reduces parasitism is documented for a variety of migratory taxa. For example, studies on wild caribou (*Rangifer tarandus*) populations have demonstrated that migratory behavior reduces parasitism by warble fly larvae (*Hypderma tarandi*). These flies lay their eggs under the skin of their host, and the larvae emerge in high abundance during the spring calving period, posing a significant risk to juvenile survival[22]. Reindeer herds that undergo a post-calving migratory period have a lower abundance of ectoparasite than herds that do not migrate, and larval abundance is negatively correlated with the distance between calving and their post-calving grounds[19]. This suggests that migratory behaviors in these mammals are adaptive, with the primary benefit being parasite avoidance while raising their calves[19].

Studies on common spiny toads (*Bufo spinosus*) have shown that hosts escape parasitism and can recover from infection during migration, demonstrating the "migratory recovery"[23] hypothesis. New infections by the aquatic amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, or *Bd*) occurred only in freshwater breeding ponds compared to during migratory and terrestrial overwintering periods[24]. Furthermore, individuals that were *Bd* positive in their breeding ponds did not show any signs of infection when recaptured during migration or at

overwintering sites[24]. This suggests that migration may offer a natural escape and remediation period, reducing the impact of *bd* infections on the host.

Several studies on monarch butterflies (*Danaus plexippus*) have presented evidence of the ways in which different migratory behaviors reduce parasitism. Experimental infections of a protozoan parasite (*Ophryocystis elektroscirrha*) in different monarch populations revealed that the population with the longest migration distance had higher survival rates and lower parasite loads after being infected compared to individuals from resident or short-distance migratory populations. The authors suggested that the high survival rate among long-distance migrants reflects selection for more resistant individuals, where individuals with more susceptible genotypes are weeded out during migration[25]. A follow-up experimental study confirmed that butterflies infected with *O. elektroscirrha* lost more mass and exhibited poor flight performance, both of which are indicators of host fitness, than uninfected hosts[26]. The observed negative effect of infection on individual flight performance supports the idea that there may be a selection for resistance to this parasite, especially in long-distance migratory monarchs[26]. Furthermore, a study that examined the prevalence of *O. elektroscirrha* during migration to southern overwintering sites found that prevalence declined progressively with southern sampling sites[27]. These findings are consistent with migratory culling and could explain the patterns of parasitism observed among monarch butterflies[27–29].

The relationship between parasitism and monarch butterfly migration was further explained by comparing the prevalence of *O. elektroscirrha* across the breeding period[27]. This study found that prevalence increased as the breeding season progressed and peaked around the onset of fall migration. This trend is consistent with the migratory escape hypothesis, which postulates that increasing parasitism in the breeding range drives the seasonal evacuation of

breeding territories. In monarchs, the transmission of *O. elektroscirrha* occurs through the use of the monarch's host plant, milkweed (*Asclepias syriaca*)[30,31]. This mechanism explains how transmission risk and parasite prevalence accumulate with time spent on the breeding grounds. Furthermore, parasite prevalence decreases across the migratory period[27] (as described above), which suggests that evacuating breeding sites may directly reduce parasitism in migratory monarch butterflies. In summary, this system is an excellent example of a system in which migration can function as an adaptive strategy that results in reduced parasitism across monarch butterflies.

Several behavioral mechanisms are documented to reduce parasitism during migration. For example, migrants may take advantage of habitat transitions to reduce parasitism, as demonstrated in salmon lice (*Lepeophtheirus salmonis*), a marine coepod sensitive to low salinity environments[32] that infects salmonids, such as the sea trout (*Salmo trutta*). During migration to freshwater breeding sites, lice-infected sea trout preferentially choose low-salinity habitats, suggesting that this is a host behavioral adaptation for reducing exposure to this ectoparasite[33,34].

All of the studies mentioned above provide evidence of how migration can facilitate the reduction of parasitism. However, the hypothesis that parasitism is an evolutionary driver of seasonal migration is challenged by a substantial number of studies that have demonstrated the negative effects of migration on host parasitism rates.

1.2 Parasitism as a consequence of seasonal migration in birds

Studies have reported elevated rates of parasitism in several migratory animals. For example, a large meta-analysis of 93 ungulate species showed that migratory species have higher parasite richness, including viruses, bacteria, protozoa, helminths, arthropods and fungi, than

resident and nomadic ungulates[35]. Another large correlative study examined nematode richness across 200 avian species[36] using data acquired from the host-parasite database hosted by the Natural History Museum of London[37]. Similar to the previous study on ungulates[35], phylogenetically controlled mixed model approaches support that migratory birds have higher nematode richness than residents[36].

In addition to parasite richness, studies that compared parasite prevalence among seasonally sympatric migratory and resident birds at nonbreeding, intermediate stopover and breeding sites tended to find higher parasite prevalence among migrants. For example, one such study compared haemosporidian (*Haemosporida*) blood parasite infections between temperate migrants in their tropical nonbreeding ranges and sympatric South American residents[38], and found a higher prevalence and richness of haemosporidian infections in overwintering migrants than in resident species[38]. Other studies conducted at overwintering sites have found greater[39–41] or similar[42,43] parasite prevalence in residents compared with migrants. At a fall stopover site, where migratory and resident barn swallows (*Hirundo rustica*) co-occur, migratory individuals host a greater abundance of bacterial lineages in the microbiome[44]. Furthermore, a comparison of three species of sparrow (*Passer*) at their sympatric breeding range in Southern Europe found an association between different migratory behaviors and haemosporidian prevalence[45]. Specifically, the fully (*P. hispaniolensis*) and partially (*P. montanus*) migratory species harbored lower parasite prevalence but greater diversity than the resident species (*P. domesticus*)[45]. Few studies have examined parasitism in migratory and resident birds at sympatric breeding sites.

Several factors might contribute to the positive relationship between parasitism and avian migration. First, migrants experience greater habitat diversity between their breeding, stop-over, and nonbreeding sites than hosts that inhabit the same range year-round. This diversity of

habitats may in turn expose migratory species to a higher diversity of parasites[1,46]. Additionally, the pre-migratory period is associated with increased host gregariousness, as migrants prioritize fattening, which provides the fuel essential for flight performance and survival[47]. The consequential increase in host density can increase the risk of transmission among migrants and residents[7,48]. Furthermore, some ectoparasites have synchronized their reproductive periods with the pre-migratory period, taking advantage of the higher transmission risk among hosts[48].

For long-distance migrants from high latitudes, increasing the migration distance involves spending nonbreeding seasons at increasingly lower latitudes. Lower latitudes are associated with greater parasitism due to an expected latitudinal diversity gradient[49,50], with tropical habitats near the equator harboring higher parasite and pathogen abundances and diversity. As a result, long-distance migrants may experience higher parasite diversity than shorter-distance migrants who winter at northern latitudes. In addition, long-distance migration poses unique physiological challenges for sustaining movement over large distances[1]. This is particularly relevant for migratory birds, which are highly mobile and have some of the longest known migration distances.

Infections not only jeopardize immediate survival during migration, but may also have long-term repercussions, affecting reproductive outcomes[8,51]. Specifically, infected migrants often stall movement at stopover sites[52] and spend time recovering from infection[53,54] or improving their body condition[55]. During spring migration, stalling can delay arrival at breeding sites[56,57], which can reduce the probability of acquiring high-quality breeding sites and reduce fecundity[57–60]. Thus, infections can have cascading effects on future reproductive success.

1.3 Reproductive costs of parasitism

The relationship between parasitism and reproductive success is a central component in the association between parasitism and migration. This relationship reflects life-history theory, which describes a trade-off between investment in reproductive effort and survival[61] due to limitations in resource acquisition. Parasitism is expected to reduce both survival[8] and reproductive success[62,63] where the activity of infection survival strategies, such as immune defense limit the host's reproductive investment[12,61,64].

The trade-off relationship between parasite defense and reproduction is empirically supported by a number of studies, including Nordling et al.'s study on collard flycatchers (*Ficedula albicollis*)[12]. This study experimentally manipulated clutch size or the number of offspring per brood as a proxy for reproductive effort. Females with larger clutches had a higher intensity of *Haemoproteus* infection at the end of the nestling feeding period and reduced immune responses after immunization with Newcastle disease virus antibodies compared to females with smaller clutches. This suggests that females with greater reproductive effort are less successful at fighting *Haemoproteus* infections and mounting strong immune responses to the Newcastle disease virus. Overall, this study experimentally demonstrates investment trade-offs between reproduction and pathogen defense.

A large body of literature underscores the heightened costs of parasitism during the reproductive phase. For example, nest parasitism can diminish survival, hatching, and fledging success in nestlings[10,65–68]. While hosts evolve strategies that are geared towards minimizing the effects of parasitism during the reproductive phase, parasites, in response, must concurrently adapt[69,70]. For example, ectoparasites can synchronize[48,71] their lifecycles with their host's reproductive period to exploit underdeveloped immune systems[72,73] utilizing horizontal and

vertical transmission at the nest[74,75]. The negative effects of parasitism during reproductive periods can carry-over to subsequent breeding attempts[10,66]. This suggests that it may be beneficial to migrate away from highly-parasitized breeding habitats[4–7] if migrants return to their breeding sites with less parasitism, benefiting reproductive success[5,7,76].

The complex relationship between migration and parasitism highlights the adaptive challenges faced by migratory species. Threats of parasitism impose selection on the evolution of physiological and behavioral strategies to mitigate risk and also shape broader evolutionary trajectories. Yet, to understand the extent of the relationship between migration and parasitism, it is imperative to consider their impact on immune defense, which has evolved over millennia to combat the threats of parasitism.

1.4 Pathogen and immune defense during migration.

If migrants harbor a higher diversity of parasites and pathogens than residents, then selection may favor adaptations that improve or maintain proper immune function to reduce fitness consequences on hosts. However, our understanding of how parasitism during migration selects for improved immune defense is incomplete.

The immune system of vertebrates includes both innate and adaptive immunity. These have divergent functions, both of which are necessary for the defense against pathogens and parasites. The components necessary for innate immune function are constitutively present at low levels in the blood, providing a rapid first-line of defense against pathogens[77]. When the innate immune system detects an antigen, the body launches an energetically costly acute-phase response to clear the infection[77,78].

Birds must budget sufficient fat, water, and protein stores to survive migration[56,79,80]. During flight, physiological stress can reduce body condition[80] and immune function[46], which

can lead to increased host susceptibility[81,82]. Eikenaar and Hegemann examined the impact of migration on immune function by comparing the microbial killing capacity (BKA) in migratory and resident blackbirds (*Turdus merula*) at an intermediate stopover site during fall migration[83]. BKA is an eco-immunological measure of innate immune function, and reductions in this measure reflect a lower ability to fight infections[84]. Migratory individuals tended to have lower BKA than residents[83], suggesting that migrants have reduced immune function, strained by flight. Similarly, experimental long-distance flight trials of European starlings[85] (*Sturnus vulgaris*) and another of Western sandpipers[86] (*Calidris mauri*) found decreased innate immune responses after long-distance flight trials. These studies conclude that migrants might reduce their investment in the innate immune system to improve their flight performance.

In contrast to the generalized innate immune response, the adaptive immune response is highly specific to the invading pathogens[87,88]. Upon recognition of an antigen, the adaptive immune system signals downstream production of antibodies that target and destroy the invading pathogen. The diversity of antibodies in the body reflects the diversity of pathogenic threats that can initiate an immune response[89,90]. Specifically, the Major Histocompatibility Complex (MHC) is the gene complex that encodes the receptors responsible for initiating the adaptive immune response[91].

The evolution of genetic diversity at the MHC is driven by pathogen-mediated selection, which drives MHC evolution[90,92], as the diversity of the gene complex is correlated with the diversity of pathogens a host can defend against. Selection at the MHC maintains genetic diversity through balancing selection, acting through heterozygote advantage, negative frequency-dependent selection, and fluctuating selection[93–95]. Furthermore, large-scale gene duplications lead to large copy numbers that diversify quickly, resulting in a large number of

unique alleles at the MHC[96,97]. Accordingly, MHC diversity is measured as the number of unique alleles and as nucleotide diversity, which represents sequence diversity across alleles[98–100].

The diversity of the MHC has been correlated with host traits, including seasonal migration[101–103]. Evidence for a relationship between MHC diversity and host migration is supported by a comparative study of MHC diversity in a migrant and a resident population of common yellowthroats, *Geothlypis trichas*[104], where migrant populations host a greater number of MHC alleles and have greater haemosporidian infection prevalence than the non-migrant population [104]. Furthermore, in a large correlative study of 34 nonpasserine species, Minias et al.[102] found that migration was associated with the strength of selection, measured as the number of nonsynonymous mutations (dN), which reflects nucleotide sequence diversity. Similarly, MHC copy number was significantly positively correlated with migration in these nonpasserine species[105]. Considering the evidence for a positive relationship between migration and parasite diversity, selection may favor diverse MHC as an adaptive response to parasitism during migration. Such adaptations could be crucial for survival and on-time arrivals during migration.

Immune responses are energetically costly, and investment in these functions is mediated by investment in other life-history traits such as reproduction[12,64], molt cycles[106], and migration[28,46]. Life-history theory suggests that fast-living species (those with short life spans and high reproductive effort) rely on nonspecific innate immunity, which is more physiologically costly than the adaptive immune system[107]. Slower-living species (those that breed less rapidly and live longer) favor less costly and more specific adaptive immunity[108]. A major component of the efficiency of the adaptive immune system is its ability to store antibodies, which facilitates a faster and more direct response to subsequent reinfections[109]. The efficiency of immunological memory may be especially valuable for slow-living migratory species that are repeatedly

exposed to different parasites throughout their annual migration to breeding and nonbreeding ranges. This is supported by studies that compared resident and migratory birds from sympatric breeding ranges and found that migration and increasing migration distance are associated with slower-paced lifestyles[110–112] (but see [113]). This is because the efficiency of the adaptive immune system is in line with that of slower-living species, which prioritize longevity and resource efficiency. If parasitism during migration selects for efficient immune responses, it seems reasonable to expect selection to maintain a diverse repertoire of adaptive immune functions in migratory animals.

In summary, if migration increases exposure to diverse parasites and pathogens, this necessitates evolutionary adaptations to defend against them. Although the innate immune system offers rapid and generalized defense against invaders, it may be temporarily suppressed during migration where the immunocompromised hosts are susceptible to new infections and the re-emergence of latent infections[46,82,114,115]. In contrast, the specificity and efficiency of the adaptive immune system may be critical for migratory species to address pathogenic threats. Investigating the immune response complements comparisons of parasitism and seasonal migration because it provides insights into how selection responds to evolutionary pressures and trade-offs that are important for migratory lifestyles.

1.5 Knowledge gaps regarding the associations between parasites and migration, and the effects of migration on the immune system.

As described in previous sections, there are multiple non-mutually exclusive explanations for the relationship between parasitism and seasonal migration. For migratory birds, empirical support for the influence of parasite avoidance as a driver of avian migration remains limited. Instead of

being the primary reason for the evolution of migration, higher parasitism may be a consequence of migration. If this is the case, parasitism poses challenges that migrating birds must adapt to.

In this dissertation, I address this knowledge gap by examining variations in ectoparasitism and immune gene diversity in migratory birds that sympatrically breed in northern latitudes. I compared ectoparasitism during the spring and fall migratory periods to assess support for the hypothesis of migratory escape, which predicts that parasitism increases during the breeding season, leading birds to migrate during the fall. If migration is indeed related to parasite avoidance, then I expect greater ectoparasitism during the fall migratory period than during spring.

Additionally, because there is conflicting evidence for both positive and negative relationships between parasitism and migration, I tested the correlation between the presence of ectoparasites during migration and the average migration distance of each host species.

Second, I assessed if MHC Class I diversity is associated with the expected pattern of differential exposure to pathogen diversity due to different migration distances, which may reflect an evolutionary strategy to mitigate the impact of parasitism on migratory birds. I sequenced an exon of the MHC-I that encodes a segment of the antigen-binding groove of the MHC receptor and compared its diversity among closely related species with different migration distances. If there is a correlation between pathogen diversity and migration distance, I also expect a correlation between MHC-I diversity and migration distance.

Seasonal migration, observed in various taxa across the animal kingdom, is not a simple behavior but involves complex genetic, environmental, and physiological traits that evolve under strict ecological and evolutionary constraints. Central to understanding this behavior is the dynamic relationship between migration and parasitism, which continues to shape evolutionary

processes. Whether migration serves primarily as a refuge from parasitism or whether it introduces its own set of parasitic challenges remains a subject of ongoing research. While the "migratory escape" hypothesis speculates that migration is an adaptive strategy to evade the harmful effects of parasites and pathogens; there are also compelling arguments suggesting that long-distance migration might lead to increased parasitism. The immune system is an integral component as it is the host's first line of defense, responding to pathogen-mediated selection that varies across different migratory behaviors This dissertation examines the associations between parasitism, migration, and immune responses. I focused on migratory birds because of the variability in their behaviors in this group, which allows us to explore the evolutionary trade-offs and strategies that are necessary for survival and reproductive success in seasonal environments.

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Chapter 2 Can Seasonal Migration Serve as a Strategy to Reduce Parasite Exposure in High-Latitude Regions?

2.1 Introduction

Seasonal migration, which is a round-trip movement between often disjunct seasonal ranges, has been hypothesized to reduce parasitism of migratory taxa[1–4]. However, there is contrasting evidence that increased parasitism is a negative consequence of migration rather than a driving force behind the evolution of migratory behaviors. Therefore, the relationship between parasitism and seasonal migration remains poorly understood.

One proposed mechanism for the hypothesis that migration reduces parasitism is known as "migratory escape," which refers to instances where migratory taxa leave breeding ranges that become increasingly infested over the breeding season[5–8]. This hypothesis is based on observations that parasite abundance tends to increase during the breeding season. Specifically, there is an increase in horizontal and vertical transmission at nesting sites[9–11], putting juveniles, who tend to be disproportionately susceptible to infections[12,13], at risk. Furthermore, hosts are more vulnerable because resource investment is diverted towards reproduction at the expense of immune defenses[14–16]. The negative effect of parasitism on reproduction and corresponding decrease in host fitness[17–19] may promote a fitness benefit to evacuate increasingly infested breeding locations to return to more favorable conditions.

Independent of the migratory escape hypothesis, migration has also been suggested to reduce transmission rate if physiological stress due to the cost of travel results in reduced fitness or the death of highly infected hosts ("migratory culling"[20,21]). In birds, there is evidence that

demanding flights decrease some axes of immune system function[22,23] suggesting that infected hosts may be especially vulnerable during migration[24].

Prior studies have demonstrated that migration may facilitate infection recovery ("migratory recovery")[25]. Habitat transitions throughout migration can promote the drop-off of certain parasites. For example, the abundance of harmful ectoparasitic[26,27] lice declined as their Mourning dove (*Zenaida macroura*) hosts progressed southward through increasingly dry climates during migration[28,29]. Complementary experimental trials suggested that decreasing humidity resulted in a sharp drop-off of ectoparasite abundance among Mourning Doves and other columbiform hosts, independent of variation in immune defense capabilities[30].

While the above studies are consistent with the hypothesis that seasonal migration reduces parasitism, they contradict a substantial number of studies that document the negative consequences of host migration on parasitism in birds. For example, migration can lead to transportation and range expansion of parasites[31–33], as well as increased parasite diversity[34–36] and susceptibility[12,24] to new or latent infections due to compromised immune function caused by physiological stress[22,24,37,38]. Infected migratory birds may need to stall movement at stopover sites to recover from poor body conditions due to infection[39–41], which could lead to delayed breeding site arrival[42–44] and reduced fecundity[44–47]. These studies suggest that increased parasitism or infection may be a cost of migration that impairs both survival and reproductive success.

Due to numerous studies that document a relationship between migration and parasitism, host-parasite relationships have been considered a central driver of the evolution of migration[3,33,48]. However, given the evidence for both positive and negative relationships between migration and parasitism, the nature of the interaction and its contribution to the

evolution of migration remains unclear. Additional non-exclusive hypotheses suggest that migration is primarily driven by resource tracking that enables breeding in regions with high seasonality, with the benefit of seasonal access to more favorable breeding sites (see[34,49–51]). In high-latitude environments, I expect that the challenges imposed by a resource-poor winter may be prominent selective forces driving migratory behavior, whereas the contribution of parasite avoidance in these types of habitats remains unknown.

Here, I tested some of the predictions (**Table 1.1**) associated with the hypothesis that migration may serve as a parasite avoidance strategy by examining the presence of mites and other ectoparasites infecting birds among 64 host species that were salvaged during the fall and spring migratory periods in the midwest region of the United States. Because the sampling location was at a northern latitude, the fall collection period followed the reproductive season and initial stages of fall migration. The spring collection period captures the presence of ectoparasites at the end of the spring migratory period, as they return to their breeding range. I evaluated the presence of ectoparasites during these seasons to test the relationship between migration and parasitism in a high-latitude environment, where I expect that avoidance of harsh winter climates is a likely driver of migration. The implications of these results in the context of evolutionary migration are discussed.

Table 2.1 Testable predictions for hypotheses regarding the relationship between seasonal migration away from the breeding range and parasitism.

Hypothesis	Expected trends	Predictions	Rational
Increasing parasitism drives migrants to evacuate breeding sites.			
	1. Parasite prevalence increases leading up to fall migration.	Ectoparasitism is greater in the fall compared to spring.	Horizontal and vertical transmission at the nest. Synchronization of host and parasite reproductive and dispersal phenology.
	2. At the onset of the migratory period, residents may have more, or similar rates of parasitism compared to migratory species.	In the fall, ectoparasitism in residents is greater than that in migratory species.	Residents remaining at increasingly infested breeding habitats may experience greater parasitism than migratory species.
Migration away from the breeding range decreases parasitism			
	3. Migratory species have fewer parasites after migration compared to before their migration.	For migrants, ectoparasitism is lower in the spring.	Escape from seasonally parasitized environments. Culling of highly infected individuals. Infection recovery during migration.
	4. Migratory species return from their migration with less parasitism compared to residents.	In the spring, ectoparasitism is lower among migratory species compared to residents.	Species that do not migrate do not lose as many parasites as migratory species after the migratory periods.
	5. Long-distance migrants are impacted by migratory culling more than short-distance migrants.	There may be an inverse relationship between migration distance and ectoparasitism in the spring.	The effects of physiological stress scales with migration distance.
Migration away from the breeding range increases parasitism			
	6. Migrants have more parasites after migration than before migration.	For migrants, ectoparasitism is greater in the spring than in the fall	Physiological effort during migration increases host susceptibility. Increased parasite diversity and abundance at lower nonbreeding latitudes.
	7. Migratory species return from their migration with greater rates of parasitism compared to residents.	In the spring, ectoparasitism is higher among migrants than residents.	Species that do not migrate do not experience the same increase in parasitism as migratory species.
	8. Long-distance migrants may be more susceptible to infection.	There is a positive relationship between migration distance and ectoparasitism at the end of spring migration.	The effect of migratory culling is greatest among long-distance migrants.

2.2 Methods

2.2.1 Presence/absence dataset.

The data used in this study were collected by B.M. O'Connor between 1964 and 2019 from salvaged bird specimens and deposited at the University of Michigan Museum of Zoology. Specimens are salvaged and donated under salvage permits held by the University of Michigan Museum of Zoology. Salvaged specimens include animals that were collected post-mortem that have typically died from natural causes or anthropogenic impacts. For birds, this often includes window collisions during migration. This study assumes that window collisions and other salvaged bird specimens represent a random sample of the population, in terms of host characteristics and parasite infection load. Specimens are processed and stored as skins, skeletons, and/or tissue samples within the collection and are accessible for research by the scientific community.

Host specimens are stored frozen prior to being examined under a dissecting microscope. Prior to processing the salvaged host, the host was combed for any detectable mites or other ectoparasites (hereafter collectively referred to as "ectoparasites"). Recovered ectoparasites were identified and classified by B.O.C and were transferred to 70–100% ethanol. Some ectoparasites embedded within the skin or quill may only be detectable with destructive sampling and are thus not obtainable with these detection methods.

Each individual host had a single entry documenting the presence or absence of ectoparasites based on four taxonomic categories (defined in **Appendix Table A.1**), including two groups of mites (Arachnida: Acariformes: Astigmatina, Prostigmata) and one group of Parasitiformes (Arachnida), including the orders Mesostigmata and Ixodida. Another group recorded the presence of infections by other ectoparasites, such as lice and fleas (Insecta). Some

hosts were infected by ectoparasites belonging to multiple taxonomic groups. Further taxonomic classifications, such as family or genus, were reported whenever possible. These data do not contain estimates of parasite load.

To test the relationship between migration and the presence of these ectoparasites, I filtered the data to include individual records from passerine birds (Order Passeriformes) where the host species was represented at least five times in our dataset. I focused on observations from hosts collected within the Great Lakes region, including Michigan, Minnesota, Illinois, Wisconsin, Ohio, and Indiana, to reduce variation in ectoparasite abundance due to geographic range. Because these data are biased towards hosts salvaged during migration and did not permit a thorough examination of their breeding and nonbreeding seasons, I removed a small number of breeding or nonbreeding specimens and limited my analysis to the fall (September, October, and November) and spring (April and May) migratory periods. I did not include any observations from nests or nestlings, or observations with missing or incomplete salvage dates.

2.2.2 Bayesian mixed statistical models.

To examine seasonal variation in the presence of mites and other ectoparasites while accounting for host ecology and phylogenetic divergence between species pairs, I used Bayesian mixed models incorporating Markov chain Monte Carlo (MCMC) simulations implemented in the R package *brms*[52].

The primary response variable was the presence or absence of four taxonomic groups (Astigmatina, Prostigmata, Parasitiformes, and other ectoparasites; see **Appendix Table A.1**). Each response group was modeled using the "Bernoulli" distribution[53], as appropriate for binary response variables. The Bernoulli distribution reports the log odds of effect sizes and can be

converted to describe the probability of ectoparasite presence as a more directly interpretable value. All models were run using 4 chains, each with 8000 iterations including 4000 as warmup.

To control for phylogenetic effects, I used a phylogenetic correlation matrix from derived from a host phylogeny provided by birdtree.org[54] as a random effect. I assessed model convergence using $\hat{R}$[52,55] ($\hat{R}$=1), effective sample size [52,55] (ESS > 1000) and by examining trace plots. I reported the log-odds estimate, 95% credible intervals (CI), and the effective sample size for each fixed and random effect. The effect of predictors was reported as significant if the 95% CI did not include zero. I plotted the median estimate and 95% CI using forest plots to visualize the effects of each model term. I illustrated the interactions between season and migration using the conditional_effects() function from *brms*, which reverse-transforms regression coefficients from the logit scale[52] so that effect sizes are directly interpretable as probabilities.

2.2.3 Host ecology and life history

Because parasitism is related to life history and ecological traits, such as mass[16,56], habitat[57,58], reproduction[59–61], immune investment[14,16], molt cycles[62–64], and migration[3,65], I compiled host ecological and life-history traits from Birds of the World online[66] (**Appendix A.2**) and examined the contribution of these traits as fixed effects to control for their influence in these data.

To represent ecological variation, I examined the effects of habitat type, nest type, diet and foraging behavior, each of which have been correlated with parasitism (traits defined in **Appendix Table A.3**). Different habitat types have distinct associations with parasitism. For instance, aquatic [63,67], humid[30,59], and ground[32] habitats are associated with higher parasite burdens than terrestrial, dry, and arboreal environments. Additionally, ectoparasite transmission is facilitated by close social contact on the nest[9,10] and nesting habitat can influence exposure[59].

Specifically, while cavity nests are associated with increased transmission[68,69], exposed open nest types have more ectoparasitism compared to enclosed cavity nests[70,71]. Additionally, I included foraging behavior to represent the intersection of diet and habitat variation[72]. Aerial foraging describes insectivores that occupy aerial habitats compared to species that forage for arthropods among foliage or in tree bark. Ground foraging is associated with larger tick loads compared to habitats above ground level[32]. It's important to note that species are categorized based on habitat and behavior observed in the breeding range. These classifications do not consider potential shifts in diet and habitat throughout the annual cycle.

I used four traits to represent life history variation, including the season of pre-basic molt[62], brood number[73], clutch size[73,74], and mass[16,19,56]. Birds differentially invest in energetically demanding life history functions throughout the year according to distinct periods: reproduction, molt, and migration[75,76]. Most species from northern latitudes typically undergo their molt cycle at the end of the summer, coinciding with post-breeding and prior to fall migration, although variations in this strategy exist[77]. In addition, molt-migrants simultaneously invest in molting and flight performance, which can compromise parasite defense during migration[77,78]. In order to consider the impact of the hosts' molt strategy on ectoparasitism, I classified each species' molt cycle based on the season in which the pre-basic molt occurs and the presence or absence of molt migration according to Pageau et al.[78]. Brood number and clutch size were chosen to represent reproductive effort[79]. Mass can be a proxy for numerous life history parameters, such as immune function[16], reproductive effort[79] and parasitism[80], justifying inclusion of this trait. Species average body mass was assigned following Dunning [66,81].

I included species' breeding and nonbreeding latitudes, by calculating the centroid of a species' seasonal graphic ranges using data hosted by BirdLife International and NatureServe[82].

Breeding and wintering range latitudes are important confounding variables considering an expected association between lower latitudes and increasing parasite diversity and abundance[83]. Higher latitude breeding ranges are associated with longer migration distances, as these habitats are necessarily further away from milder climate nonbreeding sites, and with shorter reproductive seasons[75]. Additionally, breeding latitude could also be interpreted as indicative of how far individuals had progressed on fall migration or how close they were to their putative breeding grounds in spring migration and vice versa with nonbreeding latitude compared to our sampling location, though I note that I lack data on individual migration distance.

I estimated migration status, as migratory or resident per species, and species average migration distance as the distance from the centroid of the breeding range to the centroid of the wintering range. Additionally, I categorized migration distance as resident (0 km), short- (< 2500 km), medium- (2500 – 4500 km), and long- (>4500 km) distance as ordered factors to compare model fit between the two calculations of migration distance. I standardized all continuous predictors (clutch size, brood number, migration distance and mass) to a standard deviation of one to yield comparable effect sizes.

2.2.4 Model development

I developed two generalized linear mixed model equations through a model selection approach using leave-one-out cross-validation[84] (LOOCV) to identify the best fitting models to test the predictions for ectoparasite presence that are described above (**Table 2.1**). LOOCV estimates model performance by resampling the data and omitting one data point at a time and then computes the accuracy of the model to predict the omitted data point. The main advantage of LOOCV is that it uses all available data to estimate model performance which can yield less biased results compared to Bayesian methods, such as AIC.

For both model equations, I used similar approaches to determine the best fitting model. I fist determined the most appropriate combination of predictors related to migration by comparing model performance of different combinations of season, migratory status (equation 1) or migration distance (equation 2), breeding latitude, nonbreeding latitude, and the interaction of season with each of the other three. Each combination of migratory predictors was compared to a null model that included only the effects of phylogenetic variance and the best fitting model was determined using loo_compare[84].

After determining the best fitting combination of migration and season, I then added one additional fixed effect predictor that represented host ecological (habitat type, nest type, diet and foraging behavior) or life history traits (season of pre-basic molt, clutch size, brood size and mass). Due to significant correlation between the ecological traits (**Table 2.2**), I retained only the single best fitting ecological trait.

Table 2.2 Chi-squared correlations between host ecological traits.

The chi-squared test statistic and corresponding p-value are reported within each respective cell for unique combinations of traits. Chi-squared tests were implemented in R using the chisq.test function with simulate.p.value set to TRUE.

Ecological Traits	Habitat broad	Nest type	Diet	Foraging behavior
Habitat broad	x			
Nest type	228.26, 4.9×10^{-4}	x		
Diet	163.02, 4.9×10^{-4}	108.32, 4.9×10^{-4}	x	
Foraging behavior	654.21, 4.9×10^{-4}	398.23, 4.9×10^{-4}	216.28, 4.9×10^{-4}	x

The first model was developed to test the effect of being migratory (compared to resident) on ectoparasitism depending on the migratory season (fall or spring) and is utilized to test some of the predictions related to each of the three hypotheses described in **Table 2.1** (Predictions 1-4, 5-6). As a hypothesis, migratory escape does not directly describe an association between parasitism and migration distance, just that the existence of migration is driven by parasite avoidance. To reflect this component of migratory escape, I included host

migration status (migratory or resident) rather than migration distance as the primary predictor for this model. Further, the migratory escape hypothesis described escape from the breeding range, I focus my interpretation of results on ectoparasitism in the fall. In contrast, when considering the two hypotheses, that migration increases or decreases parasitism, I focus my interpretation of the results from this model equation on samples from spring migration, which represents ectoparasitism upon arrival at or near the breeding range.

The second model tests the relationship between migration distance and ectoparasite presence. I adjusted this model equation to consider the effect of migration distance, rather than migratory status, which is captured in the first model equation. I considered both continuous and categorical estimates of migration distance within the model selection approach. The use of migration distance emphasizes the influence of physiological effort and constraint on arrival date[46,47,85] which scales with increasing migration distance[34,85]. I focused on evaluating the relationship between migration distance and ectoparasite presence during the spring season, when the differential physiological effects of varying flight distance and constraint on arrival date may be more pronounced compared departure in the fall for many species, as the sampling location is closer to their breeding grounds (the destination).

2.3 Results

After filtering, I retained 1,195 observations of 64 host species across 47 genera. In total, there were 693 and 502 records for the fall and spring migratory periods, respectively. Overall, 979 (82%) salvaged birds were infected with at least one ectoparasite, while 216 were uninfected. Astigmatina was the most prevalent group as 926 salvaged birds hosted at least one mite from this group and there were considerably fewer Parasitiformes, Prostigmata, and other ectoparasites recovered from infected hosts.

When considering all hosts and exclusively migratory hosts, there were significant differences between fall and spring prevalence among all ectoparasites (total presence) and among Astigmatina and Prostigmata mites ($p < 0.001$; **Appendix Table A.4**). Among resident hosts, only Prostigmata and Parasitiformes differed significantly between the two migratory periods. Generally, there were more infections during the spring migratory period for Astigmatina, Prostigmata, and total presence (**Figure 2.1**). In contrast, parasitiform prevalence was greater in the fall for all host groups, however, this is only significant among resident hosts ($p < 0.01$; **Appendix Table A.4**).

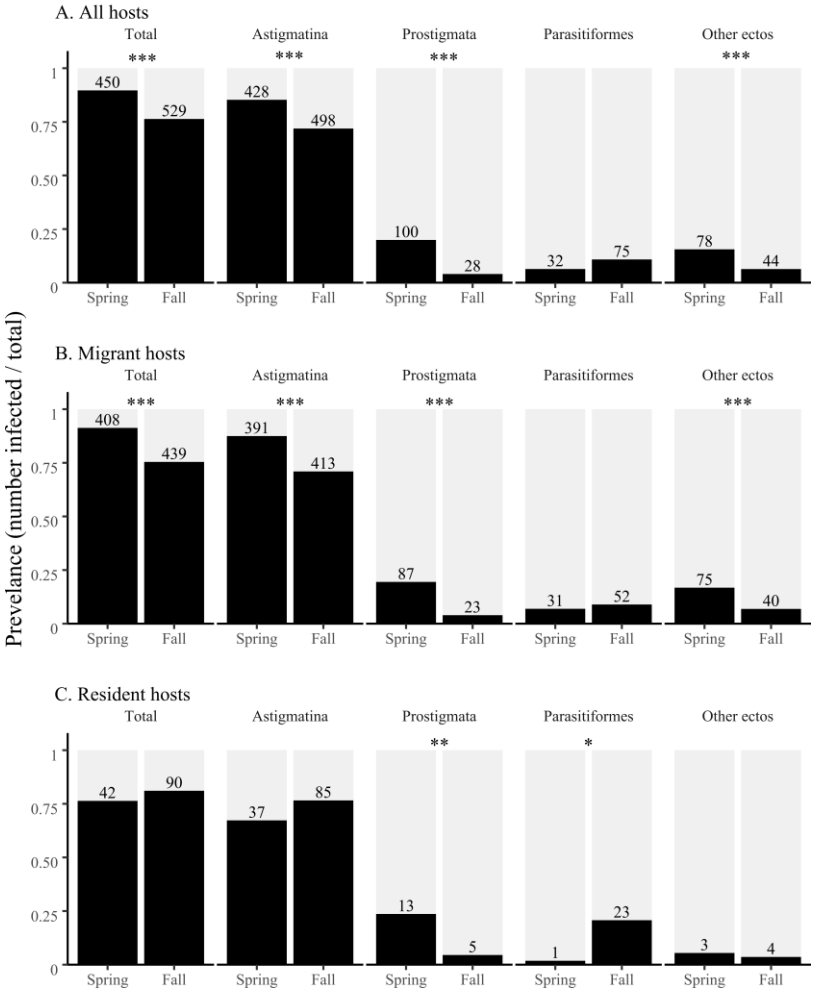

Figure 2.1 The prevalence (number of infected hosts/total number of hosts) of ectoparasites by host migratory status.

Ectoparasite prevalence among A) all host observations, B) migratory hosts, and C) resident hosts in the spring and fall migratory periods. The black and gray portions of the bars represent presence and absence, respectively. The total number of infected birds is listed within each bar. Spring: 502 (migrants:447, residents: 111); Fall: 693 (migrants: 582, residents: 55). Astrix above facets indicate significant according to Kruskal-Wallis tests (**Appendix Table A.4**; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

2.3.1 Modeling the difference in presence between migratory seasons

The final best fitting model describing the relationship between migration status and ectoparasite presence included the main effect of season, the interaction between season and migration status, nest type and breeding latitude (**Appendix Table A.3**).

The model results indicated an effect of season for four of the five response groups such that there was a lower log odds of ectoparasite presence in the fall among migratory hosts ("Fall"; **Appendix Tables B.1 –B.3, B.5**). For Parasitiformes, the log odds of presence in the fall were greater than in the spring (**Appendix Table B.4**).

Additionally, there was support for a positive effect of being a resident, and thus not migratory, on the log odds of fall ectoparasite presence ("Fall: Resident"; **Appendix Table B.2 – B.5**) for all response groups except for the total presence response ("Fall: Resident"; **Appendix Table B.1**). The significant interaction term with a negative effect (Fall: Resident; **Figure 2.2A,B,D, and E**) indicates that the change in log-odds between spring and fall among migratory species is greater than the difference in log-odds between spring and fall among resident species. Model results also revealed a significant positive effect of ground nesting that is unique to Astigmatina mites (**Figure 2.2B**) and no evidence for a significant effect of any other covariate.

After converting the log odds of ectoparasite presence to the probability scale, the probability of ectoparasite presence among migrants is between 18 and 25% lower in fall (**Table 2.2**) compared to spring. Among resident hosts, there was an effect of season on Prostigmata, in

which probability of infection by these mites was 15% lower in the fall; whereas these results support the opposite pattern in Parasitiformes, where the log odds in the fall was 63% greater (**Table 2.2**) in the spring. Otherwise, in resident birds, ectoparasite prevalence was similar among the fall and spring for total presence, Astigmatina, and other ectoparasites (**Figure 2.2A,B,E**).

Table 2.3 The change in the log odds of ectoparasite presence in the fall compared to the spring for migratory and resident hosts.

Parameter estimates, standard deviation, and credible intervals (CI) are reported for each host grouping (see **Appendix B** for detailed model results). Log odds with significant credible intervals were transformed to a probability (Pr) using the following equation: exp(log odds) / (1 + exp(log odds) and are interpreted as the difference in the probability of infection by the respective response variable in the fall compared with the probability in the spring. Negative log odds indicate that the probability of infection is lower (-) in fall than in spring. The log odds of migrants and residents were determined using the same model with residents and migrants as the reference category, respectively. Significant credible intervals are bolded.

	Log odds	SD	CIlow	CIhigh	ESS	Pr in fall
Migratory						
Total presence	-1.46	0.23	**-1.93**	**-1.01**	16974	(-) 0.19
Astigmatina	-1.34	0.21	**-1.75**	**-0.94**	19471	(-) 0.21
Prostigmata	-1.49	0.26	**-2.01**	**-0.99**	29316	(-) 0.18
Parasitiformes	0.53	0.25	**0.04**	**1.03**	27122	0.63
Other ectoparasites	-1.16	0.23	**-1.62**	**-0.7**	29631	(-) 0.24
Resident						
Total presence	-0.11	0.42	-0.93	0.74	18577	
Astigmatina	0.2	0.4	-0.57	0.99	26330	
Prostigmata	-1.76	0.5	**-2.8**	**-0.82**	24306	(-) 0.15
Parasitiformes	2.24	0.63	**1.29**	**3.79**	24306	0.9
Other ectoparasites	0.28	0.64	-0.94	1.59	20751	

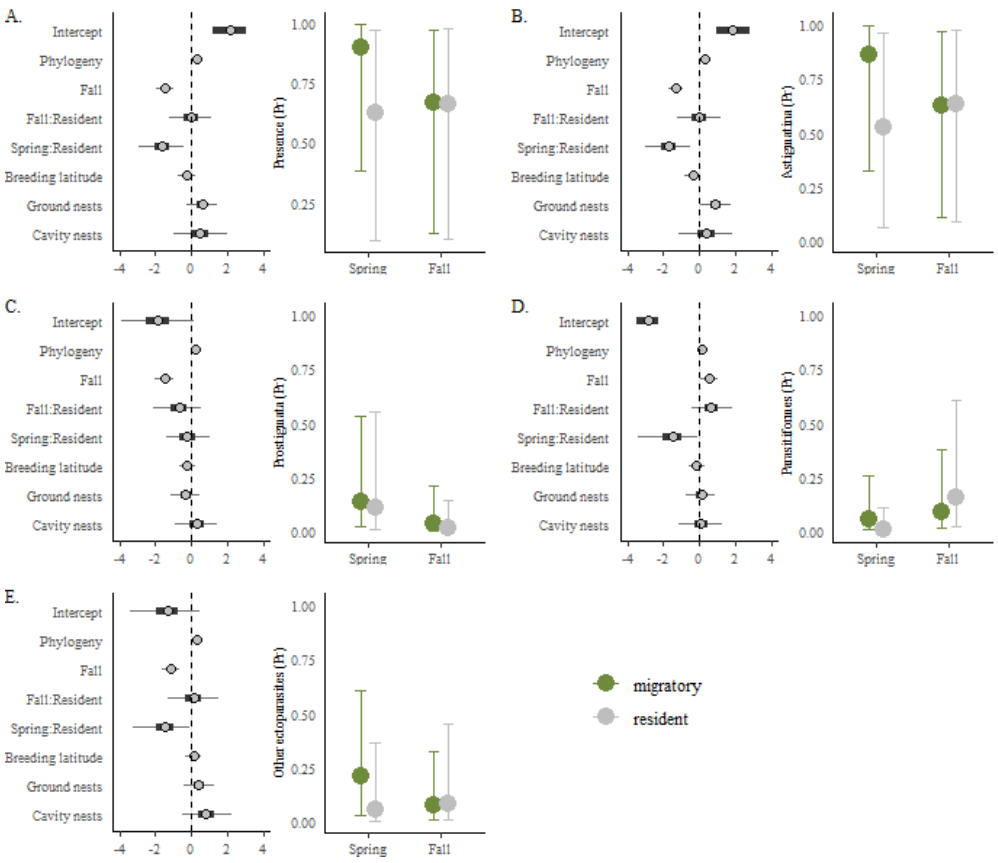

Figure 2.2 Forest plots of model one regression coefficients (left) with corresponding conditional effects plots (right) that illustrate the interaction between season and migration status for each ectoparasite group.

For the forest plots, the points represent the median values, the thick bars mark the 50% CI and thin lines mark the 95% CI, which reflect the chance that the true population value falls within this interval. CIs that did not include zero (dashed line) were interpreted as significant. Conditional effects plots illustrate the interaction between season and migration status. The x-axis represents indicates the season, and the y-axis represents the probability (0 – 1) of ectoparasite presence, which was converted from the log-odds of model results. Error bars illustrate the 95% CI. Reference categories: spring and migratory.

Modeling the relationship between ectoparasitism and migration distance during spring migration

The second model equation is designed to test the correlation between ectoparasite presence and migration distance during the spring migratory periods. Similar to the first model, the best fitting model equation included the main effect of season, the interaction between season and migration distance, and nest type and breeding latitude as fixed effects. The model that implemented continuous values for migration distance better fit these data than categorical models, thus I only

report results from continuous models. These results demonstrate a positive effect of increasing migration distance on ectoparasite presence in spring for the response groups Astigmatina, and total presence (**Figure 2.3; Appendix Tables C1 and C2**). The log odds effect size describing the change in log odds of ectoparasitism per unit increase in migration distance is provided in **Table 2.4**.

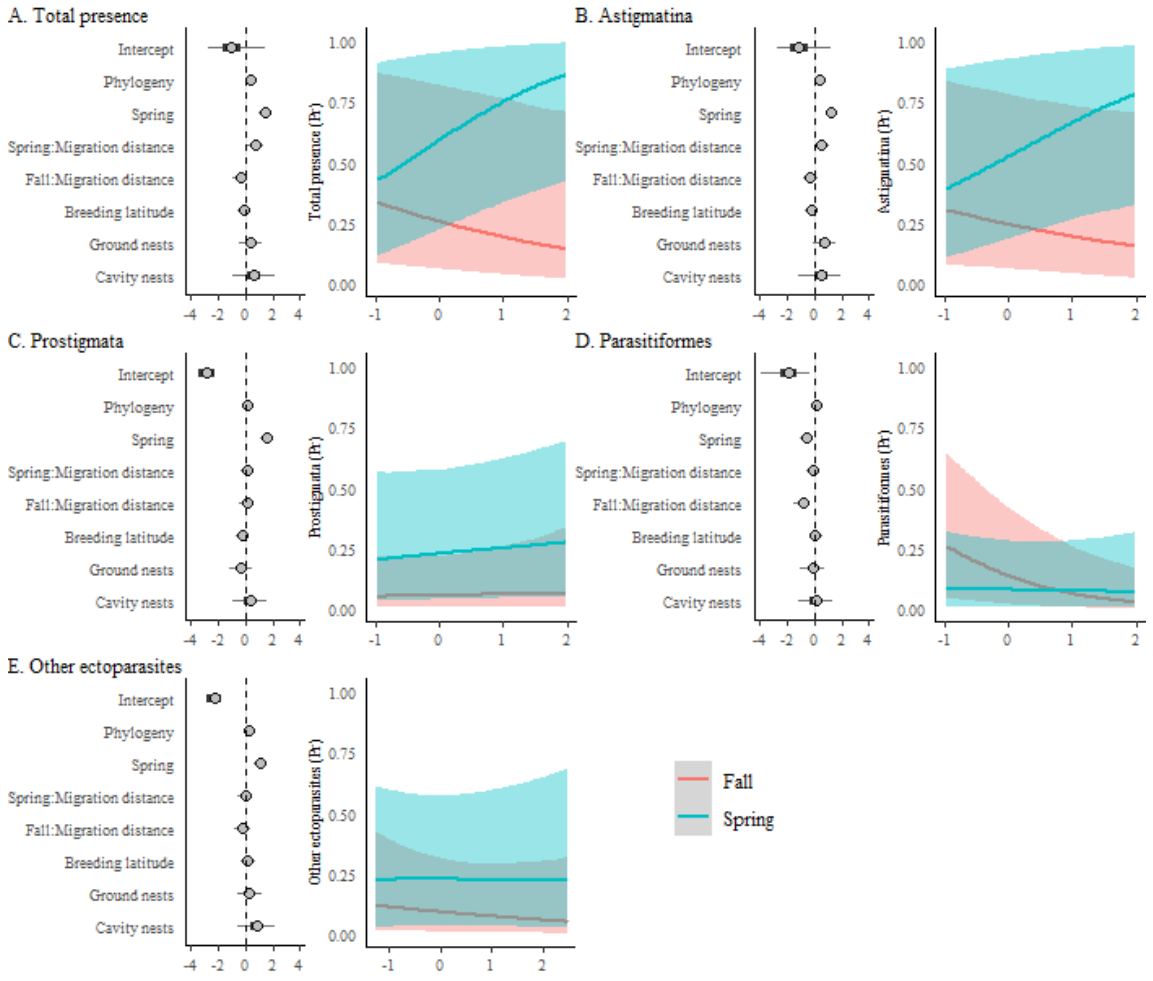

Figure 2.4 Forest plots of model two regression coefficients (left) with corresponding conditional effects plots (right) that illustrate the interaction between season and migration distance (continuous) for each ectoparasite grouping.

In the forest plots, the points represent the median values, the thick bars mark the 50% CI and thin lines mark the 95% CI, which reflect the chance that the true population value falls within this interval. CIs that did not include zero (dashed line) were interpreted as significant. Conditional effects plots illustrate the interaction between season and migration distance. The x-axis represents increasing migration distance after it has been scaled to a mean of zero and standard deviation of 1. The y-axis represents the probability (0 – 1) of ectoparasite presence, which was converted from the log-odds of model results. Error bars illustrate the 95% CI. Reference category: fall

Table 2.4 Model results describing the interaction between season and migration distance for each ectoparasite group.

The log odds parameter estimates, standard errors, and credible intervals for the log odds of the interaction between season and migration distance are provided (see **Appendix B** for detailed model results). The reported log odds represent the change in the presence of ectoparasite per unit increase in migration distance. Negative log odds indicate a negative correlation between migration distance and the probability of ectoparasite infections. Significant credible intervals are bolded.

	Log odds	SD	CI_{low}	CI_{high}	ESS
Total presence	0.71	0.33	**0.09**	**1.37**	6693
Astigmatina	0.57	0.29	**0.00**	**1.15**	5303
Prostigmata	0.14	0.22	-0.29	0.57	12189
Parasitiformes	-0.07	0.26	-0.61	0.43	13449
Other ectoparasites	0.00	0.26	-0.51	0.51	13502

Additional trends in this dataset are evident among plots of prevalence (**Figure 2.3**), although these are not supported with model results.

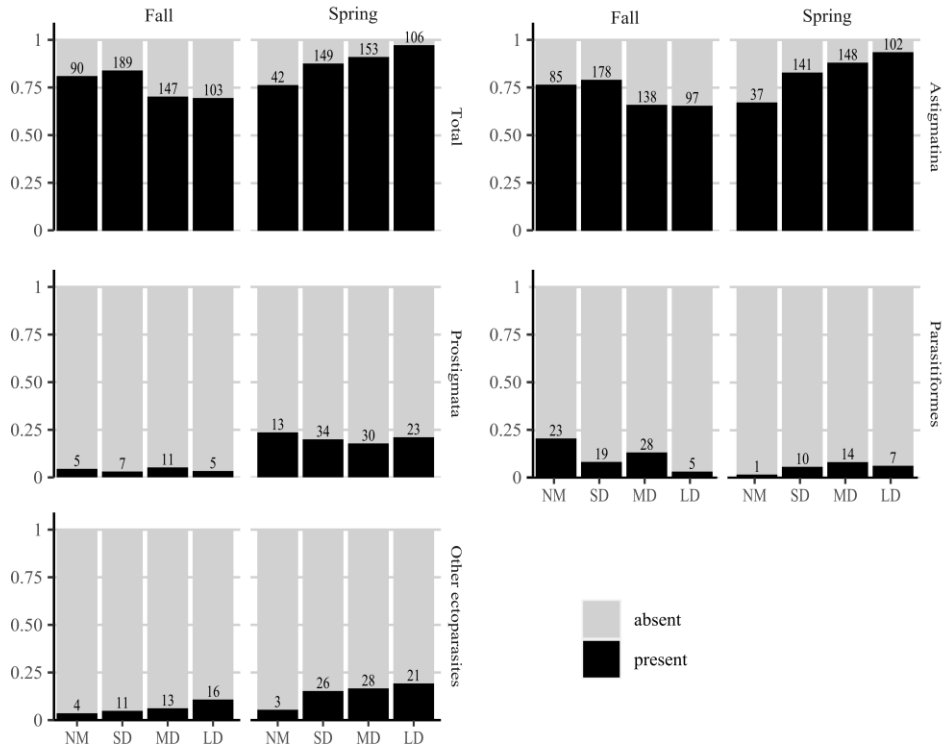

Figure 2.3 The prevalence (number of infected hosts/total number of hosts) of ectoparasites during the fall and spring migratory periods by host migration distance.

The black and gray portions of the bars represent presence and absence, respectively. The total number of birds infected with at least one ectoparasite of the respective group was reported within the corresponding bar. The x axis groups hosts by migration distance including resident (NM), short-distance (SD), medium-distance (MD) and long-distance (LD).

2.4 Discussion

I found significant differences between fall and spring seasons in all five ectoparasite groups (**Figure 2.1**, **Appendix Table A.4**) and the nature of that relationship varied across migratory and resident birds and across different ectoparasite groups. To further assess the effect of migratory behavior on these patterns, I employed Bayesian mixed models to control for host

ecological and life history traits that covary with parasitism and migration (see detailed model results in **Appendices B** and **C**) as well as host phylogenetic relatedness. Model results reveal a significant interaction between season and migration status, with minimal support for the migratory escape hypothesis (**Figure 2.2**,**Table 2.2**). Instead, these results support all three predictions (**Table 2.1**) associated with the hypothesis that migration increases ectoparasitism compared to residential lifestyles. I conclude that these results provide strong evidence for a direct association between migratory behavior and increased ectoparasitism.

2.4.1 Migratory escape is unlikely to drive seasonal migration in this system.

This study evaluated ectoparasite presence during the fall and spring migratory periods in migratory and resident birds to test for support for migratory escape. This hypothesis argues that increasing parasite prevalence throughout the breeding period drives migratory birds to evacuate their breeding range in search of more favorable conditions[3,86] and predicts that ectoparasite presence should be greater in the fall compared to the spring and especially for migratory birds (Prediction one, **Table 2.1**). Contrary to these expectations, these data indicated a lower log odds of ectoparasite presence in the fall among migratory hosts ("Fall"; **Table 2.2, Appendix B**) for all ectoparasites, Astigmatina, Prostigmata and other non-arachnid ectoparasites. Specifically, there is 18 – 25% less ectoparasitism in the fall than in the spring for migratory hosts (**Table 2.2**) independent of confounding effects of phylogeny and other host traits. In resident hosts, there was an effect of season on the log odds of Prostigmata, in which probability of infection by these mites was 15% lower in the fall.

Migratory escape may predict that parasitism is higher among residents that do not migrate (**Table 2.1**, prediction two), as was found among migratory and resident caribou in Norway[86]. These data do not support that prediction and instead reveal that ectoparasitism is

similar among migrants and residents in the fall (**Figure 2.2**), which may reflect similarities in exposure across shared breeding habitats. While this result does not directly support migratory escape, it does not contradict the hypothesis either.

Overall, the evidence for migratory escape in these data is sparse. Parasitiformes is the only group with greater ectoparasitism in the fall than in the spring (**Figure 2.2**), in line with the predictions associated with migratory escape. Further, resident species, who have not evacuated their breeding ranges, have more ectoparasites than those who are migrating in the fall; which is consistent with prediction two for migratory escape (**Table 2.1**). Parasitiformes are blood-feeding obligate parasites that include ticks and mites, whereas the other orders of parasites I studied include nasal, respiratory, quill mites. If selection against infections by ticks and mesostigmatid mites is sufficiently strong, then it is possible that avoidance of this specific group selects for migratory behavior. However, I conclude that this is unlikely because migratory escape would predict that dramatic increases in ectoparasitism across the breeding periods would select for migration, whereas this is instead observed across resident hosts (**Figure 2.2**).

In this study, I did not test the effect of host age on these data. First-year individuals tend to be disproportionately parasitized compared to adult hosts, which is reflected in the migratory escape hypothesis. Despite this expectation, ectoparasitism is lower in the fall suggesting that despite the influx of susceptible juveniles in the fall, ectoparasitism is still greater following the migratory period.

In summary, these results demonstrate a significant relationship between migratory behavior and ectoparasite presence. The nature of this effect is inconsistent with the expectations imposed by migratory escape and thus there I did not find support for the role of migratory escape as a driver of seasonal migration in this temperate study system.

2.4.2 A positive association between migration and ectoparasitism

I tested whether migration increased or decreased ectoparasite infection accordingly with predictions three–eight (**Table 2.1**). In this study, the two sampling periods are representative of departure (fall) and arrival (spring) at or near breeding sites. Differences between fall and spring ectoparasite presence will reflect the compounding impact of the migratory and nonbreeding periods on parasitism.

The results presented here demonstrate a general increase in parasitism between fall departure to spring arrival (**Figure 2.1A**). This pattern is evident among migratory hosts; whereas among residents, there are fewer differences in ectoparasitism between the fall and spring migratory periods (**Figure 2.1C**, **Table 2.2**). Further, among four of the five ectoparasite groups model results suggest that the seasonal change in log odds among migrants and residents is significantly different (Fall: Resident; **Figure 2.2**, **Appendix B**) supporting the notion that migratory behaviors do influence parasitism[33].

In line with predictions three and four that correspond to the hypothesis that migration increases exposure to ectoparasitism (**Table 2.1**), migratory species tended to have more ectoparasites in the spring compared to the fall whereas this was not observed among resident hosts (**Figure 2.2**, **Table 2.2**). This indicates that residential lifestyles do not experience the same rate of increased ectoparasitism during the overwintering period as migratory lifestyles.

Bayesian mixed models report significant positive relationships between migration distance and ectoparasitism in the spring for Astigmatina and total ectoparasite presence (**Figures 2.3A,B**; **Table 2.3**). These results are consistent with prediction five corresponding to the hypothesis that migration increases ectoparasitism. This hypothesis is supported by studies that have demonstrated a tradeoff between long-distance flight and parasite defense, possibly due

to greater physiological stress[22,24,37,87]. This pattern could also reflect the fact that long-distance migrants in this study system necessarily winter at more equatorial nonbreeding ranges that tend to have greater parasite diversity and abundances[83,88]. If parasitism experienced during the nonbreeding period persists across long distance flight, then this could also contribute to the higher rates of parasitism during spring migration. Another possible driver of these patterns could be that long-distance migrants may be more reluctant to stall migration to recover from infection at stopovers[41,89] because of selection for early arrival that greatly improves reproductive success[34,85].

In summary, these results support all three predictions posited by the hypothesis that seasonal migration increases ectoparasitism experienced by the host. Thus, I conclude that among avian species that breed at northern latitudes, migration away from the breeding range increase ectoparasitism compared to non-migratory, residential lifestyles.

2.4.3 Considering the effect of host-parasite relationships and ecology on migration in a seasonal environment

The different mite species and other ectoparasites reported in this study exhibit a range of interactions including mutualistic, commensal, and parasitic relationships with their avian hosts (**Appendix Table A.1**). For example, mutualistic feather mites, Astigmatina, consume lipidic secretions [90,91] from the host uropygial gland, which, in excess, reduce heat retention from plumage[92]. Furthermore, there is a negative relationship between feather mites and the bacteria that they consume on eggshells in the nest[93]. Infections by these bacteria reduce hatching success[94] suggesting an additional mechanism by which feather mites may act as mutualists. The mutualistic or commensal relationships between feather mites and their hosts may explain the higher rate of prevalence for this group compared to all others in this dataset (**Figure 2.3)**. If

feather mites improve host condition, the presence of these mites could provide a selective benefit that increases with migration distance, especially given their role in feather maintenance[9,95]. However, high loads of feather mites may be detrimental[96] to hosts suggesting limits to the benefits of these mites.

In contrast to feather mites, the other groups of mites in this study are parasitic. Specifically, Prostigmata mites (also known as "sucking mites") have a large gnathstoma, which includes mouthparts that are adapted to grab or pierce their host[97]. This suborder of mites includes quill mites in the family Syringophilidae, which are obligate and permanent parasites of birds, that feed on live tissue fluids from their hosts[97,98]. These mites cause inflammation and can eventually result in feather loss[98], although the full extent of their impact on hosts is unknown. The lower occurrence of Prostigmata compared to Astigmatina may reflect selection for defense and avoidance mechanisms among their hosts. However, because the statistical models for this group do not support a relationship with migration distance in the spring or fall, there is no evidence that migration could serve as a mechanism for avoidance of these ectoparasites.

The superorder, Parasitiformes comprises parasitic ticks and mites that live on and/or feed off their avian hosts and/or transmit harmful pathogens [97,99,100], such as tick-borne equine encephalitis[101] or Lyme disease[102,103]. Specifically, the northern waterfowl mite (Mesostigmata: *Ornithornyssus sylvarum*) is one of the most significant ectoparasitic threats to wild birds and domestic poultry[104]. They are permanent blood feeding ectoparasites that can result in inflammation, anemia, reduced egg production and even death by exsanguination[104,105]. While I expected strong selection against infections of Parasitiformes in the host, these data provide support for migration as a response to these selective forces. Seasonal variation in the presence

of Parasitiformes is consistent with migratory escape (**Figure 2.1**,**Table 2.3**); however, upon comparison with resident birds, this theory does not hold.

When modeling these data, I found that ecological traits, such as diet, habitat type, and feeding behaviors and life history traits, such as the season of prebasic molt, mass, clutch size, and brood number did not improve model fit. This suggests that these host traits are not effective at explaining variation in ectoparasitism despite an abundance of literature supporting their influence on rates of ectoparasitism[62–64,57,58 73,74].

2.5 Conclusion

I examined ectoparasite presence among birds that breed in high-latitude, seasonal environments to examine the contribution of parasitism to the evolution of migration. Contrary to the expectations of migratory escape, I report that the presence of mites and other ectoparasites infecting their avian hosts is lower during the fall migratory period than during spring. Thus, I concluded that there is no evidence for migratory escape in this high-latitude seasonal environment and instead, conclude that migratory behaviors result in increased ectoparasitism among avian hosts. Further, the positive relationship between ectoparasite presence and migration distance in total ectoparasite and Astigmatina mites and supports the hypothesis that increased parasitism is associated with migration. Taken together, our results suggest that parasitism is unlikely to drive the evolution of migration in seasonal environments with harsh winter conditions and instead represents a consequence of the evolution of migration.

Appendix A: Ectoparasites

Table 2.5 Definitions of response variables used to model the presence of ectosymbionts.

Known symbiotic relationships (C: commensal, P: parasitic, M: mutualistic) with avian hosts are indicated next to the family name[97,106–110]. Some families display multiple types of relationships based on species and degree of infestation.

Response variable	Taxonomy	Examples
Total presence: any mite, flea, louse, or tick on a host	Classes Arachnida and Insecta	See below
Astigmatina: feather and skin mites	Class: Arachnida Superorder: Acariformes Order: Sarcoptiformes Unranked: Astigmatina	Analgidae (C-P), Proctophyllodidae (M, C, P), Trouessartiidae (M, C), Dermoglyphidae (C, P), Xolalgidae (C), Epidermoptidae (P), Gabuciniidae (C), Dermationidae (P), Psoroptoididae (C), Pteronyssidae (P), Laminosioptidae (C)
Prostigmata: “sucking mites”	Class: Arachnida Superorder: Acariformes Order: Trombidiformes Suborder: Prostigmata	Trombiculidae (P), Erythraeidae (P), Harpirhynchidae (P), Syringophilidae (C, P), Cheyletidae (P), Ereynetidae (C)
Parasitiformes: parasitic mites and ticks	Class: Arachnida Superorder: Parasitiformes Orders: Mesostigmata and Ixodida	Ixodidae (P), Macronyssidae (P), Rhinonyssidae (P), Dermanyssidae (P)
Other parasites: lice and fleas	Class: Insecta Orders: Diptera and Psocodea	Ricinidae (P), Hippoboscidae (P)

Table 2.6 Species-specific categorical ecological traits.

The number of species (sp) and number of individuals (N) represented by each category are provided.

Trait	Category	sp	N
Habitat type			
	Forests	52	1050
	Grasslands	5	43
	Aquatic	2	20
	Towns	3	82
Nest type			
	Cavity	10	147
	Ground	15	282
	Shrub	14	336
	Tree	24	430
Diet			
	Fruit	1	36
	Insects	46	810
	Omnivore	5	83
	Seeds	10	266
Foraging behavior			
	Aerial Foraging	3	25
	Bark Forager	4	54
	Foliage Gleaner	28	585
	Ground Forager	27	539
Pre-basic molt			
	Breeding	51	969
	winter	4	69
	molt-migrant	3	157
Migratory status			
	Migratory	56	1029
	Resident	6	166
Migration distance			
	NM	6	166
	SD	20	395
	MD	24	377
	LD	18	257
Season			
	Fall	61	693
	Spring	55	502

Table 2.7 Descriptions of model specifications for each hypothesis implemented using the R package, brms.

The corresponding hypotheses and predictions (numbered according to **Table 2.1**) are provided. The variable phylo.name was used as a random effect and refers to the scientific name corresponding to the phylogenetic covariance matrix. Five response variables representing different groupings of ectoparasites (total presence, Astigmatina, Prostigmata, Parasitiformes, and other ectoparasites; see Appendix **Table A.2** for definitions) were modeled independently. Interaction terms are designated using a colon between interacting predictors. Migration distance was modeled as a continuous (migration distance) and a categorical (migration categorical) predictor.

Hypothesis	Predictions	Model
Increasing infection risk drives migrants to leave breeding sites	1	Presence ~ season + migration status + season:migration status + nest type + breeding latitude (scaled) + (1 | gr(phylo.name)
Migration increases parasitism	2 -3	Presence ~ season + migration distance (continuous, scaled) + season:migration distance + nest type + breeding latitude (scaled) + (1 | gr(phylo.name)
Migration decreases parasitism	4-5	Presence ~ season + migration distance (categorical) + season:migration distance (categorical) + nest type + breeding latitude (scaled) + (1 | gr(phylo.name)

Table 2.8 Results of non-parametric Kruskal-Wallis tests generated using the kruskal_test() function from the rstatix library[111].

Kruskal-Wallis tests evaluated the association between ectoparasite presence and migratory season (fall or spring) for five groups (total presence, Astigmatina, Prostigmata, Parasitiformes, and other ectoparasites), independently, for various host categories (all hosts, Migrant, and Resident). The table includes the total number of hosts sampled for each category (N), the test statistic, and the corresponding statistical significance. The false discovery rate correction[112] was applied to each *p*-value to account for false positives (type I errors) that arise when conducting a large number of tests. *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$

Host	N	Ectoparasite group	Statistic	*p*	
All hosts	1195	Total presence	33.9	$1.73e^{-08}$	***
		Astigmatina	29.2	$1.64e^{-07}$	***
		Prostigmata	75.1	$6.69e^{-17}$	***
		Parasitiformes	6.5	0.01	
		Other ectoparasites	25.8	$8.04e^{-07}$	***
Migrant	1029	Total Presence	42.5	$3.50e^{-10}$	***
		Astigmatina	39.4	$1.31e^{-09}$	***
		Prostigmata	62.1	$2.45e^{-14}$	***
		Parasitiformes	1.1	0.34	
		Other ectoparasites	24.0	$1.80e^{-06}$	***
Resident	166	Total Presence	0.3	0.66	
		Astigmatina	1.2	0.34	
		Prostigmata	12.0	0.001	**
		Parasitiformes	9.2	0.004	**
		Other ectoparasites	0.02	0.88	

Appendix B: Migratory Escape GLMM

Table 2.9 Model equation one results testing the association between total ectoparasite infections and season (fall or spring) based on the host's migratory status (migratory or resident) using an interaction between season and migratory status.

The log odds, standard deviation (SD), upper and lower bounds of the 95% credible interval (CI), and the effective sample size (ESS) are reported. The intercept refers to the log-odds of presence when all predictors are at the reference level (fall). Effects are considered significant and bolded if the CI does not cross zero. The model equation was as follows: Presence ~ season + season:migratory status + nest type + breeding latitude (scaled) + (1 | phylo.name).

Total Presence	Log odds	SD	CI_{low}	CI_{high}	ESS
Phylogenetic variance	0.33	0.05	**0.23**	**0.44**	4903
Intercept	2.15	1.38	-0.49	4.95	6817
Fall	-1.46	0.23	**-1.93**	**-1.01**	16974
Fall:Resident	-0.04	0.6	-1.23	1.13	8895
Spring:Resident	-1.65	0.62	**-2.9**	**-0.44**	9553
Breeding latitude	-0.28	0.24	-0.75	0.18	7862
Cavity nest	0.46	0.75	-1.03	2	9381
Ground nest	0.61	0.44	-0.24	1.47	9435

Table 2.10 Model equation one results testing the association between Astigmatina infections and season (fall or spring) based on the host's migratory status (migratory or resident) using an interaction between season and migratory status.

The log odds, standard deviation (SD), upper and lower bounds of the 95% credible interval (CI), and the effective sample size (ESS) are reported. The intercept refers to the log-odds of presence when all predictors are at the reference level (fall). Effects are considered significant and bolded if the CI does not cross zero. The model equation wa Used by the fires as follows: Astigmatina ~ season + season:migratory status + nest type + breeding latitude (scaled) + (1 | phylo.name).

Astigmata	Log odds	SD	CI_{low}	CI_{high}	ESS
Phylogenetic variance	0.33	0.05	**0.24**	**0.44**	5736
Intercept	1.86	1.39	-0.75	4.72	7529
Fall	-1.34	0.21	**-1.75**	**-0.94**	19471
Fall:Resident	0.01	0.61	-1.21	1.2	9910
Spring:Resident	-1.74	0.62	**-3**	**-0.52**	10542
Breeding latitude	-0.36	0.23	-0.82	0.09	8857
Cavity nest	0.38	0.74	-1.11	1.83	10558
Ground nest	0.89	0.43	**0.07**	**1.75**	11024

Table 2.11 Results of model equation one that tested the association between Prostigmata infections and season (fall or spring) based on the host's migratory status (migratory or resident) using an interaction between season and migratory status.

The log odds, standard deviation (SD), upper and lower bounds of the 95% credible interval (CI), and the effective sample size (ESS) are reported. The intercept refers to the log-odds of presence when all predictors are at the reference level (fall). Effects are considered significant and bolded if the CI does not cross zero. The model equation was as follows: Prostigmata ~ season + season:migratory status + nest type + breeding latitude (scaled) + (1 | phylo.name).

Prostigmata	Log odds	SD	CI_{low}	CI_{high}	ESS
Phylogenetic variance	0.2	0.05	**0.12**	**0.31**	5644
Intercept	-1.88	0.99	-3.88	0.12	10077
Fall	-1.49	0.26	**-2.01**	**-0.99**	29316
Fall:Resident	-0.72	0.67	-2.09	0.53	14442
Spring:Resident	-0.22	0.62	-1.37	1.05	14055
Breeding latitude	-0.25	0.22	-0.67	0.19	13532
Cavity nest	0.29	0.6	-0.89	1.47	16033
Ground nest	-0.33	0.4	-1.12	0.44	16007

Table 2.12 Results of model equation one that tested the association between Parasitiformes infections and season (fall or spring) based on the host's migratory status (migratory or resident) using an interaction between season and migratory status.

The log odds, standard deviation (SD), upper and lower bounds of the 95% credible interval (CI), and the effective sample size (ESS) are reported. The intercept refers to the log-odds of presence when all predictors are at the reference level (fall). Effects are considered significant and bolded if the CI does not cross zero. The model equation was as follows: Parasitiformes ~ season + season:migratory status + nest type + breeding latitude (scaled) + (1 | phylo.name).

Parasitiformes	Log odds	SD	CI_{low}	CI_{high}	ESS
Phylogenetic variance	0.18	0.05	**0.09**	**0.3**	5217
Intercept	-2.88	0.93	**-4.8**	**-1.07**	11578
Fall	0.53	0.25	**0.04**	**1.03**	27122
Fall:Resident	0.66	0.58	-0.43	1.85	15733
Spring:Resident	-1.55	0.82	**-3.37**	**-0.11**	22982
Fall:Resident	0.66	0.58	-0.43	1.85	15733
Breeding latitude	-0.15	0.22	-0.57	0.3	12725
Cavity nest	0.08	0.6	-1.12	1.26	15645

Table 2.13 Results of model equation one that tested the association between non-Arachnid infections and season (fall or spring) based on the host's migratory status (migratory or resident) using an interaction between season and migratory status.

The log odds, standard deviation (SD), upper and lower bounds of the 95% credible interval (CI), and the effective sample size (ESS) are reported. The intercept refers to the log-odds of presence when all predictors are at the reference level (fall). Effects are considered significant and bolded if the CI does not cross zero. The model equation was as follows: Other ~ season + season:migratory status + nest type + breeding latitude (scaled) + (1 | phylo.name).

Other ectoparasites	Log odds	SD	CI_{low}	CI_{high}	ESS
Phylogenetic variance	0.27	0.07	**0.16**	**0.42**	4444
Intercept	-1.36	0.96	-3.41	0.44	9088
Fall	-1.16	0.23	**-1.62**	**-0.7**	29631
Fall:Resident	0.11	0.72	-1.3	1.54	16885
Spring:Resident	-1.52	0.79	**-3.24**	**-0.09**	18626
Breeding latitude	0.11	0.23	-0.33	0.58	12843
Cavity nest	0.82	0.7	-0.51	2.24	12580

Appendix C: Migration Distance GLMM

Table 2.14 Results of model equation two that tested the association between the total presence of ectoparasite infections within each season (fall or spring) based on the host's migration distance.

The log odds, standard deviation (SD), upper and lower bounds of the 95% credible interval (CI), and the effective sample size (ESS) are reported. Effects are considered significant and are bolded if the CI does not include zero. The intercept refers to the log-odds of presence when all predictors are equal to zero or at the reference level (fall). The model equation was as follows: Presence ~ season + season:migratory status + nest type + breeding latitude (scaled) + (1 | phylo.name).

Total Presence	Log odds	SD	CIlow	CIhigh	ESS
Continuous					
Phylogenetic variance	0.33	0.06	**0.24**	**0.45**	4972
Intercept	-0.95	1.05	-2.73	1.49	6549
Spring	1.45	0.23	**1.00**	**1.92**	16710
Fall:Migration distance	-0.38	0.27	-0.92	0.15	5609
Spring:Migration distance	0.71	0.33	**0.09**	**1.37**	6693
Breeding latitude	-0.09	0.26	-0.60	0.42	5858
Cavity nest	0.64	0.76	-0.89	2.14	7331
Ground nest	0.38	0.44	-0.48	1.25	8022

Table 2.15 Results of model equation two that tested the association between the presence of Astigmatina infections within each season (fall or spring) based on the host's migration distance.

The log odds, standard deviation (SD), upper and lower bounds of the 95% credible interval (CI), and the effective sample size (ESS) are reported. Effects are considered significant and are bolded if the CI does not include zero. The intercept refers to the log-odds of presence when all predictors are equal to zero or at the reference level (fall). The model equation was as follows: Astigmatina ~ season + season:migratory status + nest type + breeding latitude (scaled) + (1 | phylo.name)**.**

Astigmatina	Log odds	SD	CI_{low}	CI_{high}	ESS
Continuous					
Phylogenetic variance	0.34	0.05	**0.24**	**0.45**	4290
Intercept	-1.03	1.00	-2.72	1.25	5837
Spring	1.23	0.20	**0.84**	**1.63**	15809
Fall:Migration distance	-0.30	0.27	-0.83	0.23	4722
Spring:Migration distance	0.57	0.29	**0.00**	**1.15**	5303
Breeding latitude	-0.22	0.25	-0.73	0.26	4845
Cavity nest	0.49	0.76	-1.11	1.93	5047
Ground nest	0.70	0.43	-0.14	1.56	6592

Table 2.16 Results of model equation two that tested the association between the presence of Prostigmata infections within each season (fall or spring) based on the host's migration distance.

The log odds, standard deviation (SD), upper and lower bounds of the 95% credible interval (CI), and the effective sample size (ESS) are reported. Effects are considered significant and are bolded if the CI does not include zero. The intercept refers to the log-odds of presence when all predictors are equal to zero or at the reference level (fall). The model equation was as follows: Prostigmata ~ season + season:migratory status + nest type + breeding latitude (scaled) + (1 | phylo.name).

Prostigmata	Log odds	SD	CI_{low}	CI_{high}	ESS
Continuous					
Phylogenetic variance	0.21	0.05	**0.12**	**0.32**	5906
Intercept	-2.88	0.90	**-4.78**	**-1.29**	10983
Spring	1.58	0.24	**1.13**	**2.07**	32046
Fall:Migration distance	0.09	0.27	-0.46	0.61	14556
Spring:Migration distance	0.14	0.22	-0.29	0.57	12189
Breeding latitude	-0.24	0.22	-0.68	0.21	12415
Cavity nest	0.38	0.64	-0.93	1.60	14299
Ground nest	-0.32	0.40	-1.11	0.45	16001

Table 2.17 Results of model equation two that tested the association between the presence of Parasitiformes infections within each season (fall or spring) based on the host's migration distance.

The log odds, standard deviation (SD), upper and lower bounds of the 95% credible interval (CI), and the effective sample size (ESS) are reported. Effects are considered significant and are bolded if the CI does not include zero. The intercept refers to the log-odds of presence when all predictors are equal to zero or at the reference level (fall). The model equation was as follows: Parasitiformes ~ season + season:migratory status + nest type + breeding latitude (scaled) + (1 | phylo.name).

Parasitiformes	Log odds	SD	CI_{low}	CI_{high}	ESS
Continuous					
Phylogenetic variance	0.21	0.05	**0.12**	**0.32**	5148
Intercept	-1.91	0.89	**-3.83**	**-0.33**	11596
Spring	-0.59	0.25	**-1.09**	**-0.10**	27726
Fall:Migration distance	-0.84	0.31	**-1.49**	**-0.28**	12681
Spring:Migration distance	-0.07	0.26	-0.61	0.43	13449
Breeding latitude	0.08	0.25	-0.38	0.59	10995
Cavity nest	0.12	0.65	-1.19	1.37	13932
Ground nest	-0.09	0.43	-0.99	0.72	15626

Table 2.18 Results of model equation two that tested the association between the presence of non-Arachnida infections within each season (fall or spring) based on the host's migration distance.

The log odds, standard deviation (SD), upper and lower bounds of the 95% credible interval (CI), and the effective sample size (ESS) are reported. Effects are considered significant and are bolded if the CI does not include zero. The intercept refers to the log-odds of presence when all predictors are equal to zero or at the reference level (fall). The model equation was as follows: Other ~ season + season:migratory status + nest type + breeding latitude (scaled) + (1 | phylo.name).

Other ectoparasites	Log odds	SD	CI_{low}	CI_{high}	ESS
Continuous					
Phylogenetic variance	0.29	0.07	**0.18**	**0.44**	6350
Intercept	-2.35	0.90	**-4.40**	**-0.77**	15251
Spring	1.06	0.22	**0.64**	**1.50**	36953
Fall:Migration distance	-0.23	0.30	-0.83	0.35	15201
Spring:Migration distance	0.00	0.26	-0.51	0.51	13502
Breeding latitude	0.20	0.25	-0.30	0.70	14679
Cavity nest	0.83	0.70	-0.56	2.21	15623
Ground nest	0.32	0.44	-0.54	1.19	17641

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Chapter 3 High MHC-1 Diversity in Migratory Birds Was Not Related to Migration Distance.

3.1 Introduction

The major histocompatibility complex (MHC) is a crucial component of the adaptive immune system that aids in recognizing and combating pathogenic infections[1–3]. The MHC encodes receptor proteins that recognize intra- and extracellular antigens[4]. Upon recognition of a foreign antigen, the MHC triggers a downstream antigen-specific immune response directed at eliminating the invader[4,5]. The immune response is then faster and stronger as previous antibodies are stored as a reference because of MHC immunological memory[6]. The genetic diversity of MHC reflects the diversity of threats that can activate an immune response[7–9]. Consequently, the MHC is among the most polymorphic gene[2] regions, and its diversity is maintained through frequent gene duplications[10,11] and balancing selection[2,12,13] to sustain effective immune function.

MHC class 1 (MHC-1), which recognizes intracellular pathogens, is commonly studied in birds and generally shows high levels of diversity. For example, in passerines, MHC-1 has diversified extensively across the avian phylogeny[14]. In passerines, copy number is especially variable[14,15], such that there are lineage-specific variations in chromosome-level architecture that are not found in other avian orders[16,17]. Genetic variation at the MHC has been associated with life-history traits, such as seasonal migration[14,18–21], as this phenomenon results in complex patterns of pathogen exposure throughout the bird's geographic range[22–24].

There is abundant evidence that different axes of migratory behavior impose differential exposure to parasites. For example, migratory birds experience more diverse habitats and are similarly exposed to higher parasite and pathogen diversity[25–27] compared to residents (but see[28]). Long-distance migrants may be particularly affected by the greater parasite diversity and abundance associated with their lower latitude nonbreeding ranges[29–31] compared with nonbreeding sites at northern latitudes. Additionally, resources may divert away from the immune system in favor of flight performance during migration[32,33,] leading to immunosuppression[33,34] and infection relapse[35,36]. However, this is not ubiquitous, as experimental trials demonstrate that birds are able to maintain flight performance while fighting infection[37,38]. This suggests that migratory birds have evolved adaptive responses to compensate for the risk of parasitism.

Studies have linked specific MHC alleles to migratory behaviors[20,39] and to specific pathogens, such as malaria[40–42], that are encountered during migration to tropical nonbreeding ranges. However, it is unclear whether MHC diversity is directly mediated by migration or whether this relationship is confounded by ecological variation and host phylogenetic relatedness.

For example, the number of MHC-1 alleles is not significantly different across migratory and resident birds that breed in the Palearctic[19,43]. Instead, African residents have a greater number of MHC-1 alleles than both Palearctic residents and migrants[43]. Together, these results suggest that breeding range conditions, such as precipitation[43], may shape MHC-1 diversity more than migratory behavior. Thus, the contribution of migration to evolutionary processes that shape this gene complex remains unclear.

In this study, I tested the prediction that long-distance migration to lower-latitude overwintering location selection for greater diversity on the MHC-1. If there is an association between migratory behaviors and selection of immune system diversity, then I expect to find higher MHC-1 diversity in long-distance migrants than in short-distance migrants. To test this prediction, I sequenced MHC-1 and assessed its genetic diversity among birds that migrated different distances. Importantly, I targeted closely related passerines that breed sympatrically in the North American boreal region but migrate different distances, corresponding to different overwintering nonbreeding latitudes. This design controls for differences in breeding habitats.

As birds may function as potential zoonotic reservoirs, understanding the evolution of the immune system and its response to differential pathogen exposure across different migratory behaviors is an ongoing research priority.

3.2 Methods

3.2.1 North American boreal belt study design

I obtained MHC-1 sequences from ten species across four passerine genera that migrate to different latitudes, corresponding to different migration distances within each genus (**Table 3.1; Figure 3.1**). Specifically, I sequenced MHC-1 for three species of *Catharus* (*C. guttatus*, *C. ustulatus*, C. fuscescens), two *Regulus* (*R. satrapa*, and *R. calendula*), three *Setophaga* (*S. coronata*, *S. virens*, *S. fusca*), and two *Vireo* species (*V. solitarius* and *V. olivaceus*).

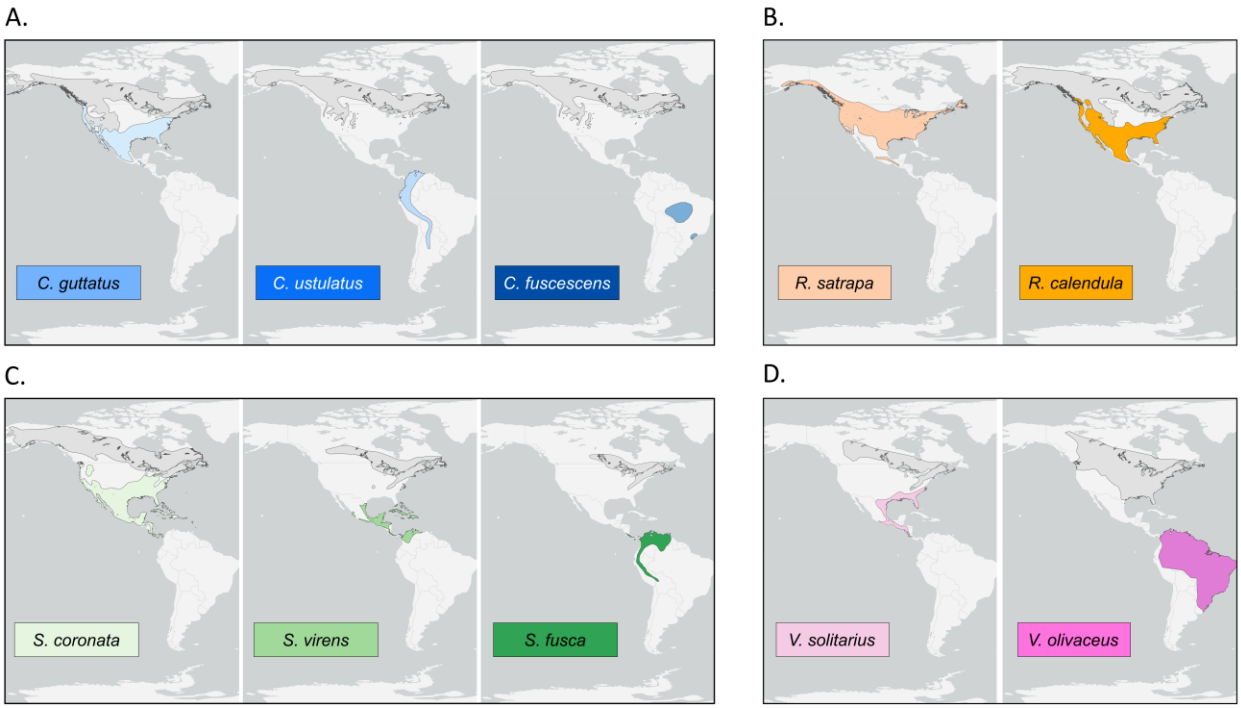

Figure 3.1 Focal taxa breeding and nonbreeding ranges.

The breeding range for each species is colored in gray, and the nonbreeding range is colored corresponding to the species label. Range maps were plotted using data from BirdLife International and NatureServe (2014) and illustrations were provided by HBW.

Table 3.1 Study taxa and host characteristics.

The average migration distance for each species was estimated as the distance between the centroid of the breeding range and that of the nonbreeding range. Migration distances were classified as short (< 2500 km), medium (2500–4500 km), and long-distance (>4500 km). The average species body mass was obtained from the CRC handbook of avian body masses (Dunning 1992) and from the Birds of North America.

Taxa	**English name**	**M_{dist} (km)**	**Category**	**Breeding Area**	**Mass (g)**
Catharus guttatus	Hermit thrush	2282	Short	8384000	31
C. ustulatus	Swainson's Thrush	7360	Long	7624000	30.8
C. fuscescens	Veery	7615	Long	3682000	31.2
Regulus satrapa	Golden-crowned kinglet	1034	Short	6210000	6.23
R. calendula	Ruby-crowned kinglet	2372	Short	9264000	6.68
Setophaga coronata	Yellow-rumped warbler	3044	Medium	10429000	12.51
S. virens	Black-throated green warbler	3707	Medium	3318000	8.8
S. fusca	Blackburnian warbler	4903	Long	2027000	9.7
Vireo solitarius	Blue-headed vireo	2759	Medium	3325000	16.6
V. olivaceus	Red-eyed vireo	5626	Long	8743000	16.7

This experimental design, contrasting closely related congeners replicated across four genera, allowed comparisons both within and across genera, while controlling for potential phylogenetic effects on MHC-1 copy number and host-parasite interactions[44–46] and life history traits that are documented to influence MHC and/or host-parasite relationships, such as major differences in body mass[14,47], habitat preferences[48,49], and diet[50,51]. *R. calendula* was recently classified as a separate monotypic genus (*Corthylio*[52]), but here, *R. calendula* and *R. satrapa* is considered a single taxonomic group as they remain their closest sympatric breeding relatives.

Given that the breeding range seems to affect the diversity of MHC in some avian systems[19,43], I focused on taxa that breed sympatrically across the North American boreal ecotone by restricting sampling to a subset of the breeding range in the boreal and hemiboreal regions of Minnesota, northern Michigan, and Manitoba (**Figure 3.2**). This minimized the potential confounding effects of differential pathogen exposure on the breeding range.

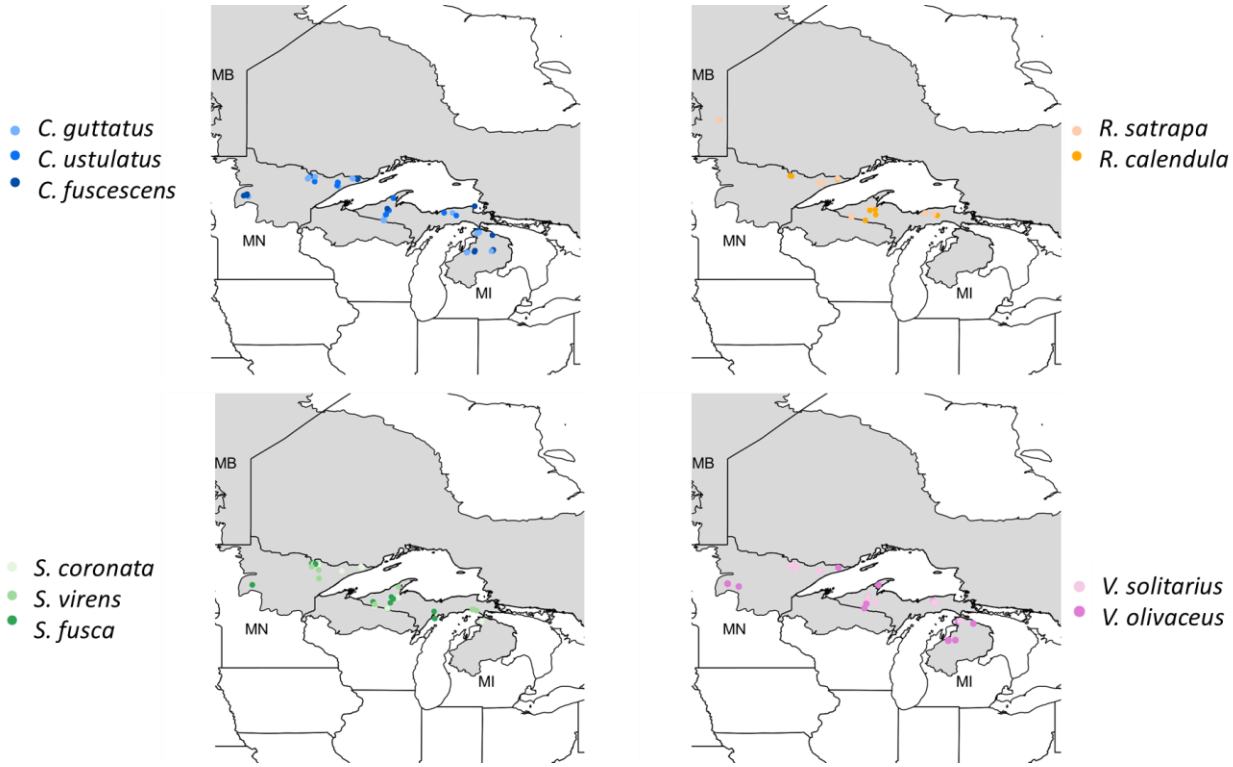

Figure 3.2 Sampling distributions of the taxa studied in this system.

The boreal ecotone is shaded gray. Colored dots indicate the approximate locations of the collection sites. The color of each dot corresponds to the species and their nonbreeding range, as illustrated in **Figure 3.1**. Most samples in this study were collected during one of the four different field exhibitions (MB,2019; MN,2017; MI,2017, 2018, 2021).

This region has a harsh winter, shorter growing season, and minimal geographic barriers[53,54]. As a result, sympatric breeders in this region are likely to experience similar climatic conditions during the breeding season, meaning that differences in pathogen exposure could be primarily driven by exposure during migration or in the nonbreeding range rather than exposure across the breeding range.

For boreal-breeding songbirds, an increasing migration distance is linked to spending the nonbreeding season in progressively tropical environments closer to the equator. Under the latitudinal diversity gradient, there is higher parasite diversity at latitudes closer to the equator[30,55]. If MHC-1 diversity in these migratory birds evolves in response to pathogen defense, there would be a positive correlation between MHC-1 diversity and seasonal migration distance. The two species of kinglet (genus *Regulus)* are both short-distance migrants (**Table 3.1**). For this specific comparison, these species may exhibit similar levels of MHC-1 diversity.

3.2.2 Sample collection and DNA extractions

I extracted DNA from muscle tissue samples of 278 individuals from four genera (89 *Catharus*, 37 *Regulus*, 93 *Setophaga*, and 60 *Vireo*) during the breeding season in Minnesota, northern Michigan, and Manitoba. The tissues were provided by the University of Michigan Museum of Zoology and the Cleveland Museum of Natural History (**Appendix Table A.1**). The samples were flash-frozen in liquid nitrogen or preserved in 95% ethanol and frozen until extraction. The samples were extracted using a Qiagen DNeasy Extraction Kit (QIAGEN, Germantown, MD, USA) following standard protocols.

3.2.3 MHC-1 exon 3 amplification and library prep

I focused on MHC-1 exon 3 because it is one of the most diverse regions of the MHC-1 locus and contains a portion of the peptide-binding region (PBR), the latter of which tends to be responsible for most of the MHC-1 functional diversity[56]. I amplified a 270 base pairs (henceforth bp) fragment that includes the entire MHC-1 exon 3. This region was sequenced using primers HN34[57] and MHCPasC1-RV[58] with Illumina overhang adapter sequences attached to the 5' end as follows: forward overhang:5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG - [HN34] and reverse overhang:5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG - [MHCPasCl-RV] (Illumina Inc., San Diego, CA, USA).

For each sample, at least two independent PCR and sequencing reactions were performed to ensure the proper identification of PCR and sequencing errors[59–61]. Each 15 µl amplicon PCR reaction contained 6.5 µl Q5 2x Hot Start high-fidelity master mix, 7.5 µM of each primer and 12.5 ng of DNA template. The PCR amplification profile was as follows:94 °C (2 min); 30 cycles of 94 °C (30s), 68 °C (45s), and 72 °C (45s), and a final extension at 72 °C (2 min). The PCR products were cleaned using MagBio HighPrep PCR clean-up beads according to the manufacturer's recommendations and then eluted with 50 µl of molecular grade water. Amplifications were validated by visualizing the PCR products on a 2% agarose gel. Following the first amplification, an additional PCR was performed to add barcodes for individual identification[62]. This reaction contained 12.5 µl of Q5 2x Hot Start high-fidelity master mix (New England Biolabs, Ipswich, MA, USA), 5 µl of molecular grade water, and 2.5 µM of each of the two indices (Nextera XT v2; Illumina Inc) and 2.5 µl cleaned PCR product. The PCR amplification profile was as follows: eight cycles at 98 °C (10 s), 55 °C (30 s) and 72 °C (15 s), with a final extension at 72 °C (5 min). I cleaned the indexed PCR products following the same

procedure described previously, but used a modified ratio of bead:PCR (0.8) to target and remove excessive primer-dimer fragments up to 300 bp in length. The final concentration of each sample was quantified after adding Illumina indices using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific/Invitrogen, Waltham, USA) and then pooled equimolar quantities of each sample to create a 4 nM library. The library was sequenced using a 300 bp paired-end Illumina MiSeq (Illumina Inc.) at the University of Michigan Advanced Genomics Core. Three libraries were necessary for 278 individuals (242, 253, and 151 reactions per library) to produce duplicate independent sequencing reactions to improve the accuracy of allele calling.

3.2.4 Filtering PCR and sequencing errors from amplicon data

Based on the workflow of Sommer et al.[61], I developed a modified decision chart for proper error identification and read filtering to identify MHC-1 alleles from amplicon sequence data. First, I used PEAR[63] to merge the reads, filter short fragments (>150), and remove low-quality bases (PHRED <15) at the 5' and 3' ends of each read. I trimmed off adapters and primer fragments from all sequences and identified putative MHC-1 exon 3 sequences by aligning reads to MHC-1 exon 3 sequences from closely related passerines using Geneious Prime v. 2022.2[64]. Subsequently, species-specific MHC-1 exon 3 consensus sequences were created using the recovered reads to improve sequence recovery from each read file. Next, identical nucleotide sequences were clustered and the number of reads for each sequence was retained using the following steps. All sequences with a read depth of one and any libraries with fewer than 500 total MHC-1 exon 3 sequences after the above filtering steps were removed. I considered 500 reads as the necessary minimum number of MHC-1 exon 3 sequences in a single library because this allowed discrimination between PCR errors and putative MHC-1 alleles in a single library. I excluded 19 samples that did not have two independent amplifications that passed these filters.

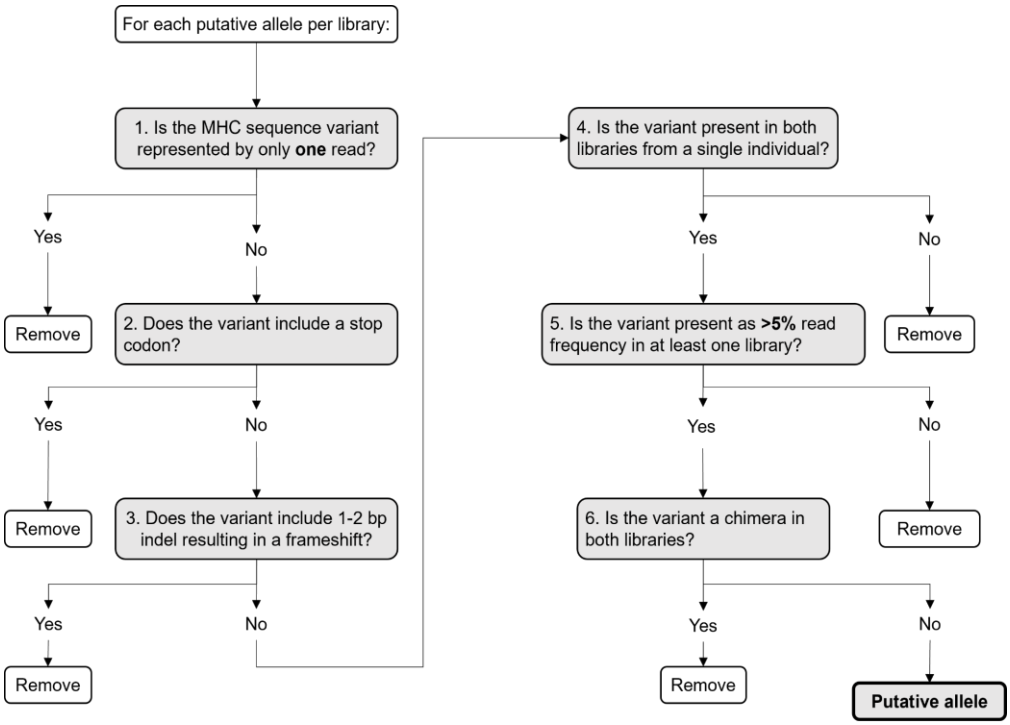

Figure 3.3 Decision tree describing process to filter errors and identify putative MHC alleles.

After each filtering step, the variants are either removed or sent to the next step. Sequences that passed all filtering parameters were considered putative alleles. The underlying workflow was modified from Sommer et al.[61].

I used the following stepwise process using the *SeqinR*[65] and *Biostrings*[66] packages in R (R Core Team 2020) to identify putative MHC alleles (**Figure 3.3**). First, I removed any sequence with an early stop codon (AGT, TAG, or TAA) or indel mutations (1-2 bp) that resulted in a frameshift mutation, as these represent sequencing errors and pseudogenes. I then compared replicate libraries for each individual and removed sequences that were not recovered from either library. Sequences that did not represent ≥5% of the total reads in at least one library for that individual were also removed. Finally, I removed chimeric sequences, which are common PCR errors[59,60,67]. Any remaining sequences were considered as putative alleles. This process was modeled using previously published methods[15,61] with a modified read-frequency threshold.

3.2.5 Estimating MHC-1 allelic and sequence diversity

I quantified multiple measures of the genetic diversity of MHC-1 exon 3 per individual and species. Specifically, allelic diversity was estimated as the total number of nucleotide (N_t) and amino acid (A_t) alleles per individual and per species, because previous studies have shown a positive correlation between the number of unique MHC-1 sequences and pathogen resistance[9,20,68–74]. Because rare MHC alleles are maintained through negative-frequency-dependent selection[2,12,13,75], I also determined the total number of private nucleotide (N_p) and amino acid (A_p) alleles (i.e., alleles that were not found in any other conspecific individual). I use private alleles as a representation of rare MHC-1 alleles, which are a key component of balancing selection and maintenance of MHC diversity[2,76,77].

To quantify sequence diversity, I calculated the mean pairwise nucleotide diversity (*pi*) for each individual and among all alleles per species using the R package *pegas* [78]. Pairwise nucleotide diversity is calculated as the proportion of nucleotide substitutions per site between two sequences and represents a measure of the genetic distance between two sequences[79,80].

3.2.6 Peptide binding region (PBR) diversity

The recognition of self- and non-self-particles by MHC is primarily mediated by the amino acids in the PBR and their physicochemical properties[81]. The physicochemical profile of each PBR sequence can describe the molecular bioactivity, including size, polarity, and charge of amino acid sequences[82,83] and is applied to examine antigen-binding interactions with MHC-1 by reflecting the diversity of antigen-binding activity among MHC-1 alleles. Supertype diversity is important to evaluate, considering that MHC supertype diversity is positively correlated with parasite resistance[84,85].

To estimate supertype diversity, I extracted and translated 19 nucleotide codons corresponding to amino acids within the PBR (**Table S2**) that have been annotated for avian

MHC-1 exon 3 sequences[58,86,87] based on homology with those in human[56] and *Gallus gallus*[8,88] sequences. I then estimated the physicochemical properties of each unique PBR sequence across all individuals using calculations of hydrophobicity (z1), steric bulk (z2), polarity (z3) and electronic effects (z4 and z5), as described by Sandberg et al.[89]. These values have been experimentally determined to reflect the physicochemical properties of peptide sequence[89].

I then used a combination of *find.clusters* and discriminate analysis of principal components (DAPC) from the R package *adegenet*[90] to cluster PBRs into distinct supertypes, following Lighten et al.[84]. First, I used the *find.clusters* function to identify the optimum number and composition of distinct MHC-1 supertype clusters using k-means clustering based on the Bayesian information criterion (BIC). Next, I used DAPC, which uses principal component analysis to reduce the dimensionality of genetic data, and then applied discriminant analysis to find linear combinations of the principal components that best differentiated between groups. DAPC identifies the most informative axes of genetic variation and uses them to create supertype clusters of alleles with similar physicochemistry[84,90].

3.2.7 Mitochondrial DNA divergence

To assess the influence of phylogenetic proximity on the MHC profile for each species, I estimated the mitochondrial divergence between each pair of species. I used previously published mtDNA genomes[91] that were constructed from all 13 concatenated mtDNA genes from a single individual in each species and aligned them using *Clustal* in Geneious[64]. To estimate pairwise mtDNA divergence, I used RAxML v.12[92] with model GTRCAT (General Time Reversible categorical) after determining this is the best model using jModelTest2[93]. This strategy approximates the time since the divergence of the species and is sufficient to assess the relative phylogenetic distance in a comparative context.

3.2.8 Statistical approach

To determine the differences in MHC-1 diversity between congeneric species, I first examined the individual counts of nucleotides, amino acids, and supertypes per genus. I tested for statistically significant differences in individual allelic diversity (number of nucleotides, amino acids, and supertype alleles per individual) and pairwise nucleotide diversity between congeneric species. I first examined each variable for normality using the Shapiro-Wilk Test implemented in R (R Core Team 2020). I used the non-parametric Wilcoxon sum rank test[94,95] and Kruskal-Wallis tests[96,97] where appropriate, using the R package *rstatix*[98] and incorporated the "false discovery rate" correction to minimize the effect of type I errors[99].

As the Wilcoxon sum rank test is sensitive to tied ranks, I adopted a resampling approach[100,101] to generate confidence intervals as a measure of the stability of significant comparisons. This was implemented by randomly resampling with replacement and then conducting a Wilcoxon rank-sum test on each resampled dataset for 1000 replicates. Dunn's test[102] was used as a post-hoc analysis for significant results from the Kruskal-Wallis test to evaluate pairwise comparisons among the triads. Additionally, the effect size was computed using Eta^2, which reflects the proportion of total variance in allele count data attributable to differences among species[103].

To examine the relationship between migration distance and MHC-1 diversity while accounting for the level of phylogenetic divergence between pairs of species, I used the Markov Chain Monte Carlo (MCMC) method implemented in the R package *MCMCglmm*[104] with a minimum of 100,000 iterations, a thinning interval of 10, and discarding the first ten percent of iterations as burn-in. The resulting mtDNA phylogeny (**Figure 3.7**) was included as a random effect to account for non-independence owing to species ancestry.

I used four different response variables to quantify the MHC-1 genetic diversity per individual, including the number of nucleotide alleles, number of amino acid alleles, number of supertypes, and *pi* (described above in **section 3.2.5**). I also examined the frequency of each supertype and the average *pi* per species for species-level comparison. I used the Poisson error distribution for all count response variables, and a Gaussian distribution to model pairwise nucleotide diversity and supertype frequency. I independently modeled three different representations of migration distance as the primary response variable:1) the distance (km) between the centroid of the breeding range and the centroid of the nonbreeding range; 2) short (< 2500 km), medium (2500–4500 km), and long-distance (>4500 km) categories 3) and the centroid latitude of the nonbreeding range (**Table 3.1**), which were obtained using data associated with BirdLife International and NatureServe[105]. I included the species' average body mass from Dunning[106] as an additional fixed effect, given that this trait is known to influence MHC diversity and/or host-pathogen relationships[14,107], as well as the nonbreeding range size (km^2) as a representative of the breadth of parasite diversity, given that population-specific dispersion across the nonbreeding destination is unknown for these taxa. I scaled all quantitative predictor and response variables to control the magnitude of differences between variables that may skew correlations and then compared the Deviance Information Criterion (DIC) of all models, of which lower values represent better model fit.

3.3 Results

Of the 278 individuals, I successfully amplified and sequenced duplicate libraries from 257 individuals. Data from 238 individuals successfully passed the filtering parameters and were genotyped for MHC-1 exon 3. The median number of MHC-1 exon 3 sequences recovered from

sequencing was 2791 sequences per individual across all libraries. The results included MHC-1 exon 3 sequences from 84 *Catharus*, 24 *Regulus*, 83 *Setophaga*, and 51 *Vireo* individuals.

3.3.1 Differences in MHC-1 diversity between and among congeneric species

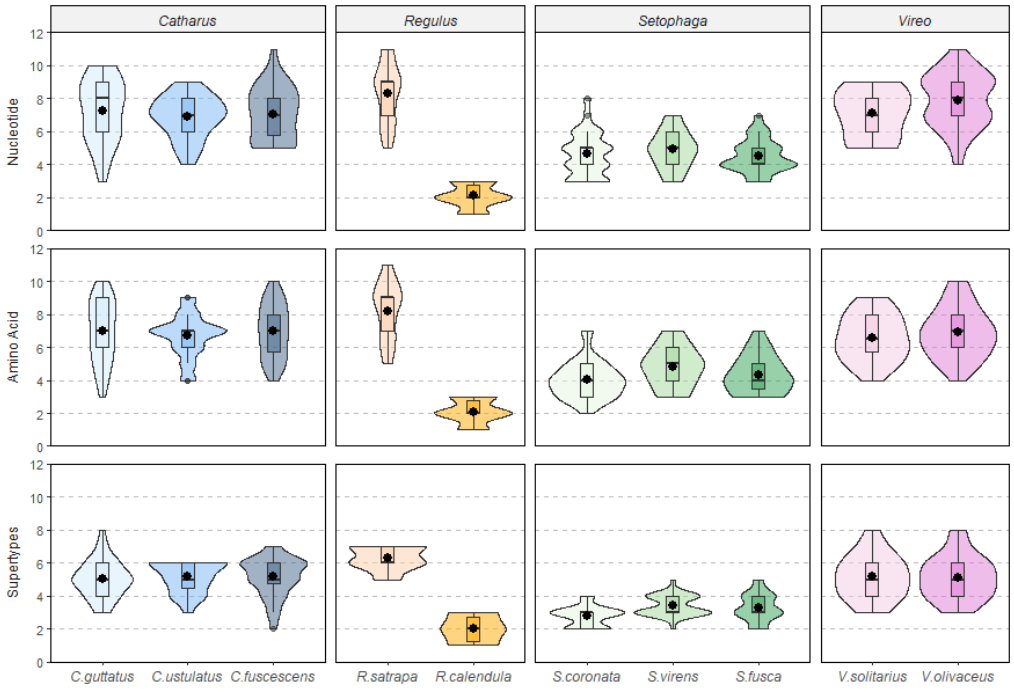

Figure 3.4 Allelic and supertype diversity.

Violin plots of the number of MHC-1 exon 3 alleles per individual for each species organized by genus. In each panel, boxplots indicate the median, interquartile interval, and range of alleles for each species. The background colors of the violin plots correspond to the species and their nonbreeding range, as illustrated in **Figure 3.1**.

I identified two *R. calendula* and 11 *C. fuscescens*, *V. olivaceus, and R. satrapa* MHC-1 exon 3 nucleotide alleles, and one *R. calendula* and 11 *R. satrapa* amino acid alleles per individual (**Figure 3.4**). The number of nucleotide alleles per species ranged from 18 (*R. satrapa*) to 143 (*V. olivaceus;* **Table 3.2**).

Table 3.2 Genetic diversity of the MHC-1 exon 3 locus per species.

The number of individuals (N), average nucleotide diversity (π), total nucleotide alleles (N_t), number of private nucleotide alleles (N_p), minimum and maximum number of nucleotide alleles per individual (N_{range}), average number of nucleotide alleles per individual (N_{ave}), total amino acid alleles (A_t), number of private amino acid alleles (A_p), minimum and maximum number of amino acid alleles per individual (A_{range}), average number of amino acid alleles

per individual (A_{ave}), total number of PBR functional groups (ST_t), and average number of functional groups per individual (ST_{ave}) are provided.

	N	**π**	**N_t**	**N_p**	**N_{range}**	**N_{ave}**	**A_t**	**A_p**	**A_{range}**	**A_{ave}**	**ST_t**	**ST_{ave}**
C. guttatus	33	0.13	122	79	3-10	7.2	103	66	3-10	7.2	12	7.2
C. ustulatus	23	0.14	97	72	4-9	7	81	58	4-9	6.9	11	6.9
C. fuscescens	28	0.14	126	95	5-11	7	114	82	5-10	7.07	11	7
R. satrapa	10	0.05	82	66	5-11	8.3	74	59	1-3	8.2	8	2.1
R. calendula	10	0.05	18	16	1-3	2.1	17	14	5-11	2.1	8	8.3
S. coronata	29	0.11	63	48	3-8	4.6	52	37	2-7	4	12	4.6
S. virens	31	0.12	56	37	3-7	4.5	45	27	3-7	4.8	11	4.9
S. fusca	23	0.13	56	41	3-7	5	50	37	3-7	4.3	12	4.5
V. solitarius	20	0.13	86	66	5-9	7.1	69	50	5-9	6.6	12	7.1
V. olivaceus	31	0.14	143	111	4-11	7.9	102	77	4-10	6.9	10	7.9

The number of nucleotide and amino acid alleles per individual did not differ significantly among congeners within *Catharus, Setophaga, and Vireo* (**Figure 3.4, Table 3.3)**. However, in the genus *Regulus, R. calendula* individuals had approximately four times more alleles than did *R. satrapa* individuals (**Table 3.3**).

Table 3.3 Intrageneric comparisons of MHC-1 allelic diversity.

The type of statistical test used is indicated by the test column (KW: Kruskal-Wallis's test, W-R-S: Wilcoxon-Rank-Sum test, Dunn: Dunn's test). The count column refers to the response variables N - nucleotide, A - amino acid, and St - supertype, as indicated in the count column. The effect size and 95% confidence interval (CI) are provided. ANOVA and Kruskal-Wallis tests use eta^2 as a measure of association, which estimates the percentage of variance in pi that can be attributed to species differences; t-tests effect size reflects the difference in means between the two groups; Wilcoxon-Rank-Sum test effect size represents the median of the difference between the two groups; and Dunn's test reports the mean rank difference between the two groups. The resulting p-values after the false discovery rate correction are presented with $p < 0.05$*, $p < 0.01$**, and $p < 0.001$***.

Genus	**test**	**Var.**	**sp_1**	**sp_2**	**n_1**	**n_2**	**df**	***p***	**Eff. size**	**CI_{low}**	**CI_{high}**
Catharus	ANOVA	N	-	-	-	-	2	0.81	0.26	0.03	4.97
Catharus	KW	A	-	-	-	-	2	0.81	$6.3e^{-03}$	$7.9e^{-04}$	0.1
Catharus	KW	ST	-	-	-	-	2	0.78	0.01	$7.2e^{-04}$	0.11
Regulus	t-test	N	*calendula*	*satrapa*	10	14	18.10	$1.2e^{-08}$ ***	-6.19	-7.34	-5.03
	t-test	A	*calendula*	*satrapa*	-	-	18.60	$1.0e^{-08}$ ***	-6.11	-7.22	-5.01
	W-R-S	ST	*calendula*	*satrapa*	-	-	-	$2.0e^{-04}$ ***	-4.00	-5.00	-4.00
Setophaga	KW	N	-	-	-	-	2	0.6	0.02	0.00	0.14
Setophaga	KW	A	-	-	-	-	2	0.06	0.09	0.01	0.26
	Dunn	A	*coronata*	*fusca*	29	23	2	0.53	6.39	-5.86	17.97
	Dunn	A	*coronata*	*virens*	29	31	2	0.02 *	16.0	4.10	26.34
	Dunn	A	*fusca*	*virens*	23	31	2	0.24	9.64	-2.72	22.23
Setophaga	KW	ST	-	-	-	-	2	0.01 *	0.16	0.05	0.32
	Dunn	ST	*coronata*	*fusca*	29	23	2	0.03*	15.4	3.59	26.88

	Dunn	ST	*coronata*	*virens*	29	31	2	$2.4e^{-03}$ **	19.7	11.14	27.93
	Dunn	ST	*fusca*	*virens*	23	31	2	0.61	4.34	-7.81	16.60
Vireo	t-test	N	*olivaceus*	*solitarius*	31	20	44.60	0.15	0.8	-0.08	1.69
	t-test	A	*olivaceus*	*solitarius*	-	-	42.80	0.61	0.3	-0.55	1.15
	W-R-S	ST	*olivaceus*	*solitarius*	-	-	-	0.95	$-4.47e^{-05}$	-1.00	1.00

MHC-1 exon 3 nucleotide diversity (*pi*) did not differ significantly between species within the same genus (**Table 3.4**; **Figure 3.5**). Mean *pi* was not significantly different among the two *Regulus* species, but *pi* for this genus was significantly lower compared to the other three genera, of which all other species have a mean *pi* between 0.11 and 0.13 (**Table 3.2**).

Table 3.4 Inter- (Genus = All) and intrageneric (*Catharus*, *Regulus*, *Setophaga*, *Vireo*) comparisons of pairwise nucleotide diversity.

The type of statistical test used is indicated by the test column (KW: Kruskal-Wallis's test, W-R-S: Wilcoxon-Rank-Sum test, Dunn: Dunn's test. I reported the effect size of each comparison and 95% confidence interval (CI). ANOVA and Kruskal-Wallis tests use eta^2 as a measure of association, which estimates the percentage of variance in pi that can be attributed to species differences; t-tests effect size reflects the difference in means between the two groups; Wilcoxon-Rank-Sum test effect size represents the median of the difference between the two groups; and Dunn's test reports the mean rank difference between the two groups. The resulting p-values after the false discovery rate correction are presented with $p < 0.05$*, $p < 0.01$**, and $p < 0.001$***.

Genus	**test**	**sp_1**	**sp_2**	**n_1**	**n_2**	**df**	***p***	**Eff. size**	**CI_{low}**	**CI_{high}**
Catharus	ANOVA	-	-	-	-	2	0.08	0.07	0.01	0.22
Regulus	t-test	*calendula*	*satrapa*	8	14	9.35	0.95	$-2.73e^{-04}$	-0.01	0.01
Setophaga	KW	-	-	-	-	2	0.3	0.03	$2.2e^{-03}$	0.17
Vireo	W-R-S	*olivaceus*	*solitarius*	31	20	-	0.95	$-6.14e^{-04}$	-0.01	$1.04e^{-03}$
All	KW	-	-	-	-	3.00	$4.3e^{-21}$ ***	0.43	0.33	0.53
	Dunn	*Catharus*	*Regulus*	84	22	-	$1.8e^{-15}$ ***	-134.9	-146	-125
	Dunn	*Catharus*	*Setophaga*	84	83	-	$8.4e^{-07}$ ***	-54.5	-70	-39
	Dunn	*Catharus*	*Vireo*	84	51	-	0.13	20.5	-1.57	42
	Dunn	*Regulus*	*Setophaga*	22	83	-	$2.6e^{-06}$ ***	80.30	71	90
	Dunn	*Regulus*	*Vireo*	22	51	-	$9.4e^{-18}$ ***	155.00	141	170
	Dunn	*Setophaga*	*Vireo*	83	51	-	$3.4e^{-09}$ ***	75.10	55	94

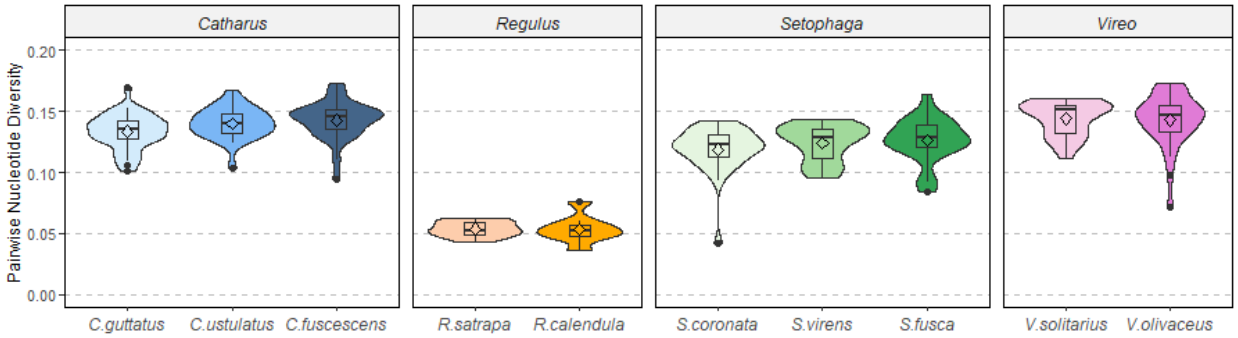

Figure 3.5 Violin plots of individual MHC-1 exon 3 pairwise nucleotide diversity (*pi*) for each species organized by genus.

Within each panel, boxplots indicate the median, interquartile range, and range of alleles for each species. The background colors of the violin plots correspond to the species and their nonbreeding range, as illustrated in **Figure 3.1**.

3.3.2 Functional supertype diversity of MHC PBRs

To estimate the functional diversity of antigen binding, the MHC-1 PBR regions were classified into amino acid supertypes, reflecting their physicochemical activity. In this study, the number of supertypes per species ranges from eight (*R. calendula* and *R. satrapa*) to 12 (*C. guttatus*, *S. coronata*, *S. fusca*, *V. solitarius*; **Table 3.2**). When comparing the supertype diversity within each genus, the number of supertypes per individual was only significantly different between the two species within the genus *Regulus* ($Median_{diff}$ = 4.0, CI: -5.0 to -4.0, p = $2.0e^{-04}$; **Table 3.3**). For each genus, most supertypes are shared between species, and only two supertypes are unique to a single genus (ST 11 in *Catharus* and ST 18 in *Setophaga*) with only one that is completely unique to a single species (ST 11 in *Catharus guttatus*; **Table 3.5**, **Figure 3.6**).

Table 3.5 The composition of MHC-1 amino acid supertypes across species.

Each row represents a unique supertype (1–25) and the number of alleles associated with each supertype per species is listed. The total number of unique supertypes per species is reported in the final row (SP total) and the total number

of supertypes across all individuals is reported in the final column (ST total). Asterisks indicate that the * supertype is found in a single species, ** supertype is found in two species, and *** supertype is found in a single genus.

ST	*C. guttatus*	*C. ustulatus*	*C. fuscescens*	*R. satrapa*	*R. calendula*	*S. coronata*	*S. virens*	*S. fusca*	*V. solitarius*	*V. olivaceus*	**Total**
1	9	18	9	0	2	0	1	3	12	2	56
2	3	0	1	2	0	3	0	2	4	11	26
3	1	0	0	6	0	15	11	8	1	6	48
4	18	12	36	0	1	3	2	2	0	2	76
5	22	14	13	6	1	3	0	2	3	15	79
6	5	2	2	14	1	1	0	2	0	12	39
7	8	1	0	6	1	4	2	1	15	6	44
8	0	0	0	1	0	1	0	0	13	3	18
9	1	0	0	10	2	2	1	1	0	2	19
10	3	1	2	3	0	2	0	3	2	8	24
11*	5	0	0	0	0	0	0	0	0	0	5
12**	0	0	3	0	0	0	0	0	0	3	6
13	5	4	7	0	0	1	0	1	0	0	18
14	2	0	0	3	1	6	7	3	14	46	82
15	0	0	0	7	1	1	1	4	2	19	35
16	0	5	6	0	0	3	5	5	9	3	36
17	1	1	0	8	0	2	10	2	0	3	27
18***	0	0	0	0	0	4	5	3	0	0	12
19	18	22	14	1	0	0	1	0	1	0	57
20	1	2	3	0	0	1	0	0	0	0	7
21	1	3	5	3	1	9	9	12	3	0	46
22	16	9	16	0	0	0	0	1	0	0	42
23	0	0	3	0	0	0	0	1	4	0	8
24	0	0	0	12	6	2	1	0	2	1	24
25	3	3	6	0	1	0	0	0	1	1	15
sp total	18	14	15	14	11	18	13	18	15	17	

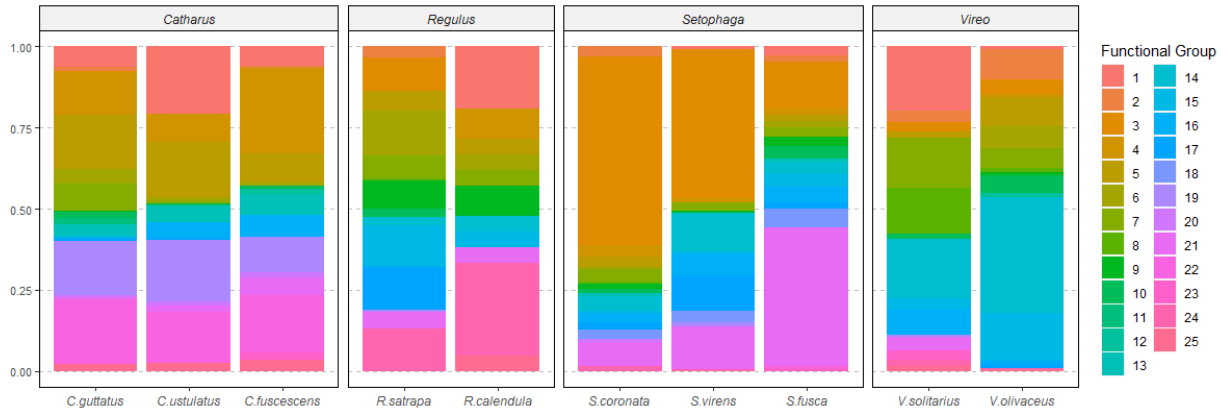

Figure 3.6 The frequency of each supertype (1-25) considering all nucleotide alleles for all individuals of each species.

The height of each colored section reflects the frequency of each ST across all the unique nucleotide sequences for each species. Functional group identity was consistent across all panels.

3.3.3 Mitochondrial DNA divergence

I estimated mtDNA divergence across all species within each genus to account for the potential effects of phylogeny on MHC-1 diversity. The topology recovered using the mtDNA genomes was consistent with that of published phylogenies (e.g., [108]). mtDNA divergence between species within each genus ranged from 0.07 to 0.2 substitutions per site **(Figure 3.7)**. *Regulus satrapa* and *R. calendula* had the greatest mtDNA divergence among the congeneric species included in this study.

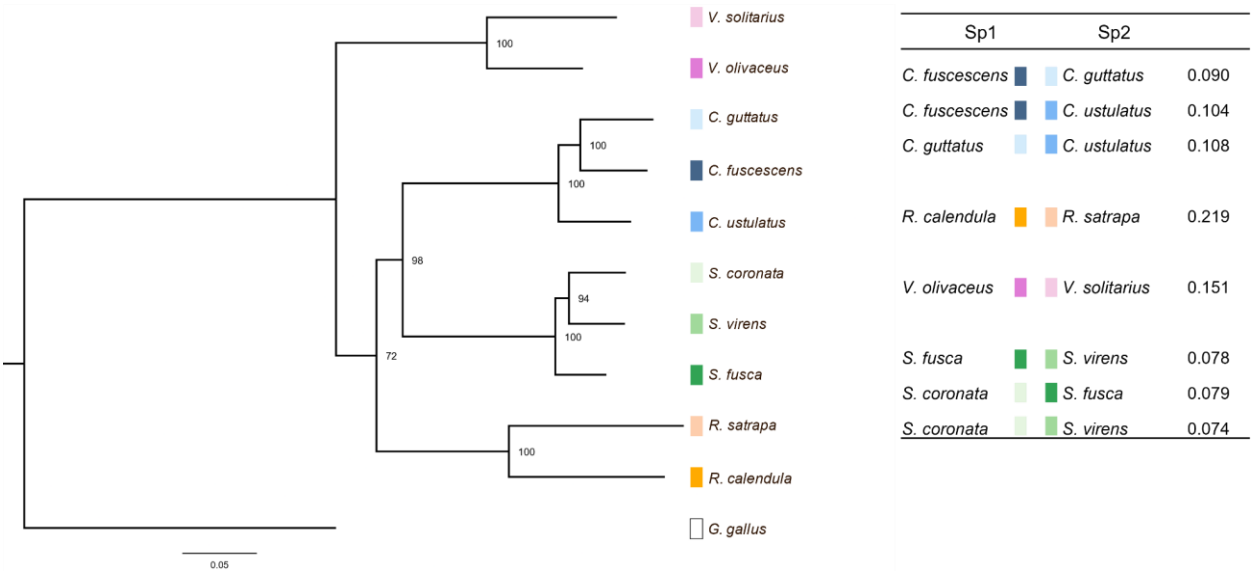

Figure 3.7 mtDNA phylogeny and divergence across 10 focal taxa.

Maximum likelihood RAxML analysis using the substitution model GTRGAMMA with *Gallus gallus* was used as the outgroup. mtDNA genomes were constructed from 13 mtDNA genes. The nodes were labeled with bootstrap support based on 500 replicates. (B) Estimated mtDNA divergence between species within each genus in the present study. In both panels, colored boxes correspond to the species and their nonbreeding range, as outlined in **Figure 3.1** (Geographic range for *G. gallus* not included).

3.3.4 No relationship between migration distance and MHC-1 diversity

There was no significant association between any measure of MHC-1 diversity, including nucleotide and amino acid allelic diversity, *pi*, supertype diversity, and migration distance (**Table 3.5**).

Table 3.6 Results from phylogenetically controlled Bayesian mixed models.

The response variables are indicated as N - nucleotide, AA - amino acid, ST - supertype, and *pi*. DIC was provided as an estimate of the model fit. The effective sample size (Neff) represents the sample size adjusted for autocorrelation. The posterior mean (PM) and 95% credible interval (CI) are provided. Significant credible intervals are in bold.

Var		DIC	N_{eff}	PM	CI
N	Phylogenetic variance	984.45	1776	1.60	**0.15 - 4.01**
	Residual		1213	0.002	0 - 0
	Intercept		15021	1.80	**1.22 - 2.36**
	Migration distance		7520	-0.03	-0.3 - 0.23
	Mass		12356	0.19	-0.32 - 0.71
AA	Phylogenetic variance	970.91	289.3	1.79	**0.17 - 4.58**
	Residual		173.4	0.00	0 - 0.01

	Intercept		4161	1.48	**0.42 - 2.57**
	Migration distance		2494	-0.00	0 - 0
	Mass		4270	0.02	-0.04 - 0.07
ST	Phylogenetic variance	879.13	92.58	1.47	**0.07 - 3.99**
	Residual		178.5	0.004	0 - 0.01
	Intercept		1613	1.23	**0.33 - 2.24**
	Migration distance		1334	0.00	0 - 0
	Mass		2279	0.01	-0.04 - 0.06
pi	Phylogenetic variance	-1278.9	413.4	0.01	**0 - 0.01**
	Residual		4999	0.00	0 - 0
	Intercept		4999	0.08	**0.02 - 0.13**
	Migration distance		4999	0.0	0 - 0
	Mass		4999	0.00	0 - 0

Regarding supertypes, the frequency of a few specific supertypes was significantly correlated with mass and/or nonbreeding range area, and none was associated with migration distance (**Table 3.4**).

3.4 Discussion

Overall, these results do not support the prediction that boreal breeding birds that migrate longer distances to lower-latitude nonbreeding grounds have a higher MHC-1 diversity than birds that migrate shorter distances. Within *Setophaga, Vireo*, and *Catharus*, there were similar levels of genetic diversity in MHC-1, including individual allelic diversity (**Table 3.2 and 3.3**; **Figure 3.4**), nucleotide diversity (**Table 3.4**; **Figure 3.5**), and supertype diversity (**Table 3.5** ; **Figure 3.6**). I report no direct evidence of an association between species-level migration distance and MHC-1 diversity in these taxa as no measure of MHC-1 genetic diversity correlated with migration distance in any statistical test. Instead, phylogeny was the strongest predictor across all models (**Table 3.5**); likely reflecting phylogenetic constraint on MHC-1 diversification.

3.4.1 Disparate allelic diversity within Regulus

Regulus was the only genus with consistent interspecific differences in allelic diversity, wherein *R. satrapa* individuals had nearly four times more MHC-1 alleles than *R. calendula* individuals (**Table 3.2**; **Figure 3.4B**). However, this does not support the hypothesized relationship between migration distance and MHC-1 diversity because *R. satrapa* has a slightly higher average nonbreeding latitude than *R. calendula,* and both species winter at relatively high latitudes[109,110] (**Figure 3.1**).

Phylogenetic distance may explain these differences, given that the two species of kinglets had the greatest mtDNA divergence (**Figure 3.7**) among all species groups. Bayesian mixed model analyses support this explanation because phylogenetic variance was the strongest predictor of MHC-1 diversity (**Table 3.6**). Owing to the greater divergence time between these two congeners, it is possible that there are differences in copy number owing to lineage-specific duplications within *R. satrapa*, that are known to occur at the MHC in birds[14,16,17]. However, the observed disparity in allelic diversity could also be explained by the low heterozygosity at MHC-1 for *R. calendula*, which would result in the recovery of fewer unique alleles per individual despite identical or similar copy numbers. This type of data cannot distinguish between differences in copy number and heterozygosity given that amplicon sequencing is not phased, meaning that homologous regions cannot be distinguished and paired.

In contrast to the disparity in allelic diversity, the mean *pi* was similar between *R. satrapa* and *R. calendula*, but significantly smaller, indicating fewer pairwise substitutions among alleles compared to the other focal taxa (**Table 3.3**; **Figure 3.5)**. It is important to note that low genetic diversity at the MHC in conjunction with low overall genetic diversity may affect the adaptability of populations in the face of pathogen-mediated selection[1,111,112]. This calls for

attention to the *R. calendula* population to evaluate the effect of low MHC diversity across this population.

However, there may be some degree of conservation in pathogen recognition, as the number and composition of supertypes (**Table 3.5**) are similar between both species despite low individual allelic diversity in *R. calendula* (**Figure 3.4**). Thus, pathogen-mediated selection may have maintained population-level MHC-1 supertype diversity in *R. calendula* despite reduced nucleotide and amino acid allelic diversity. This is consistent with studies that report that MHC supertype diversity and sufficient pathogen recognition by MHC can be maintained despite reduced genome-wide genetic diversity due to changes in effective population size[21,84]. Nonetheless, the consequences of reduced MHC diversity merit further examination of the ability of *R. calendula* to respond to pathogen-mediated selection.

3.4.2 Selective constraints on MHC-1 evolution

Selection for high MHC diversity is limited by increasing autoimmune risk, which arises when MHC misclassifies the cell's own particles as a foreign threat, resulting in a faulty immune response that destroys the host's own cells[113]. Considering that MHC-1 diversity is similar among species within each of the genera *Catharus*, *Vireo*, and *Setophaga* and that this level of allelic diversity is in line with other species of passerines[17,114,115], it is possible that MHC-1 diversity is limited by increasing autoimmune risk among these species rather than free to evolve in response to pathogen-mediated diversifying selection in this group.

3.4.3 The shared boreal breeding range and MHC-1 diversity in migratory birds

These data support the conclusions of previous studies that demonstrated an association between breeding range climate and MHC-1 diversity for migratory birds[19,43]. As this study design

minimized breeding habitat variation, it is possible that the shared breeding range drives selection for MHC diversity. This may be particularly relevant for the North American boreal belt because this system is unique in that there is an abundance of closely related taxa that breed in sympatry, which may increase the likelihood of interspecific transmission of parasites[116]. For example, in a study of the Gray-cheeked Thrush (*Catharus minimus*), the authors reported that individuals sampled in their boreal breeding ranges shared more parasite lineages with other closely related sympatric thrushes (Family: Turdidae) than those sampled in their tropical nonbreeding ranges. They suggested that interspecific transmission is most likely to occur in the breeding range due to the greater phylogenetic closeness of sympatric species in the boreal belt compared to that in their tropical nonbreeding ranges[117].

If interspecific transmission is common during periods of sympatry, then differential pathogen diversity due to migration may be homogenized among sympatric breeders[22,118–122], resulting in similar selective pressures on MHC-1 despite differences in migration, and could manifest as similar numbers and compositions of MHC-1 supertypes (**Figure 3.5**) between taxa.

3.5 Conclusion

Migratory birds are widely distributed and significantly contribute to disease dissemination. Investigation of the MHC-1 diversity in migratory birds can offer valuable insights into the mechanisms that enable these species to survive and persist in the presence of significant pathogenic challenges. I conducted an analysis to examine the relationship between the migration distance of migratory birds and the diversity of their Major Histocompatibility Complex class I (MHC-1), while also controlling for phylogenetic distance and variation in breeding range habitats among focal taxa. I found considerable MHC-1 diversity among nearly all focal taxa, and minimal differences in diversity among congeners. Furthermore, diversity

among congeners was relatively constant, indicating that migratory birds are capable of maintaining similar levels of MHC-1 diversity, regardless of their migratory distance or nonbreeding range.

Appendix A: Supertype Frequency

Table 3.7 Results from phylogenetically controlled Bayesian mixed models that test the relationship between MHC-1 supertype (ST) frequency and migration distance with the effects of mass and nonbreeding range size.

The posterior mean (PM) and 95% credible interval (CI) for each fixed effect were listed vertically by ST. Significant credible intervals are in bold.

			Migration dist.			Mass			Nonbreeding	
	PM	CI	PM	CI		PM	CI		PM	CI
Species	0.00	0 - 0								
Residual	0.01	0 - 0.01								
(Intercept)	0.04	0.03 - 0.05								
ST 1			0.04	**0.03 - 0.05**		0.02	-0.04 - 0.08		-0.03	-0.08 - 0.02
ST 2			-0.01	-0.07 - 0.05		0.00	-0.06 - 0.06		0.02	-0.03 - 0.07
ST 3			0.01	-0.05 - 0.07		-0.11	**-0.17 - -0.04**		-0.03	-0.08 - 0.02
ST 4			0.01	-0.05 - 0.07		0.05	-0.01 - 0.11		-0.01	-0.06 - 0.04
ST 5			0.01	-0.05 - 0.07		0.06	0 - 0.12		0.02	-0.03 - 0.07
ST 6			-0.01	-0.07 - 0.05		0.00	-0.06 - 0.06		0.03	-0.02 - 0.08
ST 7			-0.01	-0.07 - 0.05		0.01	-0.05 - 0.08		0.00	-0.05 - 0.05
ST 8			-0.04	-0.1 - 0.03		0.00	-0.06 - 0.07		-0.01	-0.06 - 0.04
ST 9			-0.02	-0.08 - 0.05		-0.02	-0.08 - 0.04		0.01	-0.05 - 0.06
ST 10			-0.01	-0.07 - 0.05		0.00	-0.06 - 0.06		0.01	-0.04 - 0.06
ST 11			0.01	-0.05 - 0.07		0.01	-0.06 - 0.07		0.00	-0.05 - 0.05
ST 12			-0.01	-0.07 - 0.06		0.00	-0.06 - 0.06		0.00	-0.05 - 0.05
ST 13			0.00	-0.06 - 0.07		0.02	-0.04 - 0.08		0.00	-0.06 - 0.05
ST 14			0.01	-0.06 - 0.07		-0.03	-0.09 - 0.03		0.06	**0.01 - 0.11**
ST 15			0.04	-0.02 - 0.1		-0.02	-0.08 - 0.04		0.04	-0.01 - 0.09
ST 16			0.01	-0.05 - 0.07		-0.01	-0.07 - 0.05		-0.02	-0.07 - 0.03
ST 17			0.01	-0.05 - 0.08		-0.02	-0.08 - 0.04		0.00	-0.05 - 0.05
ST 18			-0.01	-0.07 - 0.05		-0.02	-0.08 - 0.05		-0.01	-0.06 - 0.04
ST 19			0.01	-0.05 - 0.07		0.07	0.01 - 0.14		-0.01	-0.06 - 0.04
ST 20			-0.01	-0.07 - 0.05		0.00	-0.06 - 0.07		0.00	-0.05 - 0.05
ST 21			0.00	-0.06 - 0.06		-0.09	**-0.16 - -0.03**		-0.06	**-0.11 - -0.01**
ST 22			0.05	-0.01 - 0.11		0.09	**0.02 - 0.15**		-0.01	-0.06 - 0.04
ST 23			-0.01	-0.08 - 0.05		0.00	-0.06 - 0.06		-0.01	-0.06 - 0.05
ST 24			0.00	-0.06 - 0.06		-0.03	-0.09 - 0.03		0.01	-0.04 - 0.06
ST 25			-0.02	-0.08 - 0.04		0.01	-0.06 - 0.07		0.00	-0.05 - 0.05

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Chapter 4 Conclusions

In this dissertation, I have examined the relationship between parasitism and migration in migratory birds. The primary goal was to evaluate the contribution of parasitism to the evolution of migration. To this end, I examined variations in the adaptive immune response as a mechanism to address the pathogenic challenges faced during migration.

I used a study system that included both resident and migratory birds that breed sympatrically in northern latitude habitats in North America, meaning that they experience similar environmental conditions during their reproductive periods. This allows for the control of exposure during the breeding period, so that seasonal differences in parasitism are due to host-species traits, such as infection resistance or parasite exposure during the migratory and nonbreeding periods. Furthermore, species that are found in this region share similar biogeographic histories in response to the post-glacial retreat after the Last Glacial Maximum[1–3]. This resulted in recent colonization of these high-latitude breeding habitats by closely related[1–3] species with different migration distances. Therefore, comparisons of closely related species that breed in this region allow for the control of deep phylogenetic variation that influences host-pathogen relationships and long-scale genetic variation. These characteristics make this system especially valuable as a tool for systematically testing hypotheses that predict the relationship between seasonal migration and parasitism in birds.

I first described the variation in ectoparasite presence among migratory and resident birds within and across the fall and spring migratory periods (Chapter 2). Finally, I examined the

genetic variation in the adaptive immune system to evaluate its role as an adaptive response to parasitism due to migration (Chapter 3). In this chapter (Chapter 4), I summarize these results and discuss their implications for our understanding of parasitism and evolution of migratory behaviors in birds.

In Chapter 2, I found that ectoparasitism was lower in the fall than in the spring migratory period, which directly follows the breeding period. This implies a decrease in parasite presence across the breeding range and directly contradicts the expectations of migratory escape[4]. Although inconsistent with migratory escape, my results demonstrated a significant interaction between parasitism and migration. In particular, there were few seasonal differences in ectoparasitism among residents, whereas there were significant seasonal differences among migratory species between the fall and spring migratory periods. This suggests that being migratory versus being a resident influences the seasonal variation in ectoparasitism among species that breed in this region.

Analyses of ectoparasitism within the fall and spring migratory periods revealed similar levels of ectoparasitism among migrant and resident species within the fall. However, in spring, migratory species had almost universally higher rates of ectoparasitism across distinct groups of ectoparasites (Arachnida and Insecta) compared with residents, upon returning to breeding sites. These results suggest that migrants were subjected to additional parasitism between the fall and spring sampling periods (i.e., during migration to and from lower latitudes), whereas residents were not. These trends were significant, even after controlling for host ecological variation and breeding latitude. Together, this provides ample support for the hypothesis that migration increases ectoparasitism and allows me to reject the hypothesis that increasing parasitism (at least in the form of ectoparasitism) during the breeding period promotes the evolution of

migration among sympatric breeding birds. This finding is a major contribution to the understanding of the relationship between migration and parasitism among avian species.

This finding is supported by studies that have found increased endo-[5–7] and ecto-[8] parasitism in migratory birds. For example, a study that quantified the number of malaria-causing parasites, *Leucytozoon* spp., across 53 host species found a greater phylogenetic diversity of this endoparasite among migratory species compared among residents[5]. Additionally, another study of haematozoan (blood) parasites, including *Leucytozoon*, found greater diversity of these parasites among migratory waterfowl (Anseriformes) than among resident species[6]. As for ectoparasites, a study of migratory barn swallows (*Hirundo rustica*) found that the prevalence of feather mites (*Astigmatina*) increased from the post-breeding period to the pre-migratory period, and that this relationship was linked to increases in sociality during the pre-migratory period[8]. These results suggest that both endo- and ectoparasitism are influenced by migratory behavior, which is similar to the findings of my study.

Studies in non-avian systems have provided convincing evidence that ectoparasitism reduces parasitism during post-calving migrations in reindeer (*Rangifer tarandus*)[4]. There is similar evidence in insects[9,10], fish[11], and toads[12], but minimal support in birds. In birds, many studies have reported adaptations that facilitate avoidance or recovery in migratory species, such as behavioral[13,14] or habitat[15–17] shifts and stop-over recovery[18,19]. However, few studies have empirically demonstrated that these adaptations result in reduced parasitism in migratory species compared to sympatric breeding residents[20]. While theoretical studies suggest that parasitism can drive the evolution of migration in birds[21–23], there is little empirical evidence supporting this hypothesis.

Another major finding within these data was the positive correlation between migration distance and ectoparasitism. Specifically, there was a clear positive linear relationship between migration distance and total ectoparasite presence in spring. This is consistent with the idea that long-distance migrants are subject to more ectoparasites, for which there are several possible explanations. First, long-distance migrants may encounter a higher diversity of parasites at lower latitudes, which can support greater species richness and abundance (i.e., latitudinal diversity gradient[24,25]). Additionally, the stress of long-distance flight may lead to immunosusceptible hosts during the migratory periods[26,27], resulting in greater infection rates upon return to their breeding ranges. In this case, long-distance migrants may not be exposed to more ectoparasitism, but may reduce investment in avoidance or recovery compared to species with shorter migration distances. For example, migratory species may stall migration at stopover sites to recover from infection[18,19]. Stalling is costly because it can cause a delay in arrival, which shortens the length of the reproductive period[28,29], increases competition for quality breeding sites[30,31] and reduces reproductive success for long distance migrants[29,31,32]. Selection against delayed arrival may favor faster flight and reduced investment in recovery behaviors that require stalling at stopovers[18,33,34]. Although these data are not appropriate for addressing the extent to which each explanation is responsible for the observed patterns, there is substantial support for a positive correlation between ectoparasitism and migration distance. Thus, these data support the idea that migration is not a strategy for parasite avoidance, but that increased parasitism is a consequence of migratory behavior.

While migration may reduce investment in costly immune responses, it may favor investment in more efficient processes. For example, life-history theory suggests that fast-living species rely on nonspecific innate immunity, in which their activity is more physiologically

costly[35], whereas slower-living species favor investment in less costly and more specific adaptive immunity[36]. Studies that compared resident and migrant birds from sympatric breeding ranges have found that migration and increasing migration distance are associated with slower-paced lifestyles[37–39] (but see [40]). It follows that slower-paced long-distance migratory lifestyles may be associated with higher adaptive immune system function. In Chapter III, I examined genetic diversity in the MHC-1 (Major Histocompatibility complex class 1) to assess the hypothesis that differential pathogen diversity during migration can select for variation in adaptive immune function.

Migration distance and MHC-1 diversity are both expected to be correlated with pathogen diversity because of the expectation of higher blood parasite diversity[41,42], which is characteristic of lower-latitude habitats (i.e., latitudinal diversity gradient)[43–45]. Blood-parasite infections are intracellular and are fought using the MHC-1 mediated immune response[46]. Contrary to this hypothesis, I found no differences in MHC-1 diversity between closely related species with different migration distances. In addition, I found a high genetic diversity in the MHC-1 in most focal taxa compared to MHC-1 diversity in nonpasserine species. Thus, the adaptive immune system of all migratory birds may be sufficiently adapted to overcome intracellular immune challenges. This could support the importance of trade-offs between the two branches of the immune system, such that migratory birds preferentially invest in the MHC-1 mediated targeted immune responses compared to the generalized response of the innate immune system to maintain flight performance.

This interpretation is supported by experimental trials that compared flight performance, which is a common proxy for survival, between uninfected migratory great reed warblers (*Acrocephalus arundinaceus*) and conspecifics infected with a malaria-causing blood-parasite

(*Plasmodium relictum*)[47]. The flight performance of infected individuals was indistinguishable from uninfected conspecifics[47]. These findings support the conclusion that the MHC-1 mediated immune response was effective in neutralizing the impact of infection on host flight performance. This suggests that there may be selection of migratory species for high MHC-1 diversity that provides efficient immune responses to intracellular pathogens and could explain the finding of high diversity across all migratory species in my study.

Additionally, in monarch butterflies (*Danaus plexippus*), resident and long-distance migratory populations were experimentally infected with *Ophryocystis elektroscirrha*, a protozoan parasite that has detrimental effects on monarch butterfly populations. The migratory population showed high infection survival rates[48]. This implies that long-distance migrants are better adapted for survival to infection than resident and shorter-distance migratory populations, which is possible if the genotypes associated with higher parasitism are weeded out through the process of migratory culling[48]. If this type of selection occurs in migratory birds, this may explain the selection for efficient immune function in migratory birds. However, in monarchs, migration is known to decrease parasitism[49,50], whereas the opposite trend was found in this study, and it is unknown whether culling contributes to rates of parasitism among migratory birds.

In this dissertation, we found no evidence that the high adaptive immune response diversity is different among closely related birds with different migration distances. Another explanation is that increasing autoimmune risk[51] may constrain MHC-1 diversity. High MHC diversity increases autoimmune risk, because the chances that an MHC receptor may inadvertently recognize its own host as a threat and initiate an immune response that targets the host scales with increasing MHC diversity [51]. Because high MHC diversity is common among

migratory passerines, it is possible that further diversification of the MHC is restricted, rather than being free to respond to positive selection on genetic diversity. This could explain why I found similarly high MHC-1 diversity among closely related birds with different migration distances. If this is the case, there may not be differences in immune function among migratory species, or variations in immune function may be associated with other components of the immune system. Nonetheless, MHC has been independently associated with pathogen diversity[52–54] and migration[54–56], justifying the use of this gene complex as a marker of adaptive immune function in migrating birds. Additional insights could be drawn if future studies simultaneously compare the variation in intracellular blood parasites and MHC-1 diversity (e.g., [57]) in the context of bird migration.

The lack of a relationship between MHC-1 and migration distance (Chapter III) is seemingly inconsistent with the earlier finding that ectoparasitism is positively correlated with migration distance in spring (Chapter II). This contrast can be explained by the fact that MHC-1 defends against intracellular parasites[46], such as bacteria and blood parasites, and does not combat ectoparasitic infections. Thus, a strict correlation between ectoparasitism and MHC-1 is not expected, because the prevalence of ectoparasitism may not correlate with intracellular parasitism. The exception is ectoparasites that are vectors for intracellular diseases, such as *babesia*[58], which is a protozoan transmitted by ticks (Ixodes), and *Anaplasma phagocytophilum*, which is a bacterium associated with quill mites (Syringophilidae) and ticks[59]. Furthermore, our analysis of ectoparasitism evaluated its prevalence and did not consider parasite diversity. Considering that MHC genetic diversity is mediated by endoparasite diversity, there may be little association between prevalence and diversity of ectoparasites. In summary, the relationships

between migration, ectoparasitism, and MHC-1 are minimally comparable and should be interpreted carefully.

The results of this dissertation provide compelling evidence to support the relationship between migration distance and parasitism. Some studies on migratory birds have found similar correlations[60]; however, others have demonstrated that habitat variation[16], which is interconnected with migration, may drive these patterns. Similarly, studies of MHC diversity have found that habitat diversity, rather than migration distance, influences MHC diversity. In this study system, I cannot distinguish the effects of different nonbreeding habitats from the effects of different migration distances, because I only sampled birds in their breeding latitudes. For sympatric breeding birds at northern latitudes, different migration distances are associated with different nonbreeding habitats and even lower latitude nonbreeding sites. Thus, the positive relationship between migration distance and ectoparasite presence may reflect parasitism in nonbreeding habitats or the trade-off between lower immune efficiency and fast flight to breeding grounds. To understand which factor is responsible for the observed pattern, it is critical for future studies to sample birds in both breeding and nonbreeding ranges.

In conclusion, this dissertation focused on evaluating the contribution of parasitism to the evolution of migration. These studies used a system of migratory and resident birds that breed in northern latitude habitats in North America, allowing for control of parasite exposure during the breeding period. Together, I report a positive relationship between migration distance and parasitism and show that high immune diversity is characteristic of migratory birds, independent of migration distance. This implies that migratory birds are well-adapted to fight pathogenic infections. These results suggest that parasitism does not drive the evolution of migration, instead, it is a consequence that migrants are adapted to overcome.

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