Disentangling the effects of host relatedness and elevation on haemosporidian parasite turnover in a clade of songbirds

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Abstract. The persistence of a parasite species in an ecological community is determined both by its environmental tolerance and host breadth. The relative contribution of these niche characteristics to parasite community structure is challenging to parse because host persistence is also a consequence of extrinsic environmental factors. We investigated haemosporidian parasites (genera Plasmodium, Leucocytozoon, and Haemoproteus) in a clade of avian hosts in eastern North America. Species in this clade of Catharus thrushes occupy specific elevational bands in a non-phylogenetically determined manner. This allowed us to tease apart the effects of host relatedness and elevation on parasite community structure, diversity, and infection prevalence. We screened blood and tissue samples from 414 adult birds from four mountain ranges in the Appalachian Highlands for blood parasites using a cytochrome-b-nested PCR protocol and identified parasite lineages by sequencing. We found an overall infection prevalence of 88.4% and identified a total of 38 parasite lineages including six novel lineages. Parasite community patterns varied by genus. Host relatedness rather than elevational zone predicted the beta turnover and phyllobeta turnover of Leucocytozoon parasites, indicating that closely related rather than geographically proximate host species had more similar parasites. This pattern was not seen in Plasmodium parasites because the diversity of this parasite genus varied considerably in the high elevational zones among mountain ranges, that is, a sky-island effect. Haemoproteus parasites were rare in this study system. Our study suggests that the mechanisms that underlie community structuring vary between haemosporidian genera due to differences in the degree of host sharing among lineages.

Key words: avian malaria; beta diversity; Catharus; community turnover; elevational turnover; Haemospororida; host relatedness; Leucocytozoon; Plasmodium.
INTRODUCTION

Characterizing parasite community structure is fundamental to predicting the potential impact of global change on host–parasite interactions. The presence of a parasite species in a particular ecological community is largely determined by its environmental tolerances, but also the specificity with which it infects host species in that community (Krasnov and Poulin 2010, Poulin and Krasnov 2010). For example, vector-transmitted parasites must survive outside their thermoregulated vertebrate hosts (Gage et al. 2008) and ectoparasites spend their entire life cycle outside of their hosts’ body (Poulin and Krasnov 2010). The niche of these parasite groups is, thus, strongly determined by environmental tolerances. There is considerable variation in environmental tolerances of different parasite species (Lindsay and Birley 1996, Santiago-Alarcon et al. 2012), affecting both the geographic range and the infection prevalence of parasites across that range (Sehgal et al. 2011, Zamora-Vilchis et al. 2012). Parasites also display a broad spectrum of host specialization strategies, and specificity itself can be affected by the variability in environmental conditions, such as rainfall seasonality and wetness of dry seasons, encountered by parasites throughout their hosts’ ranges (Fecchio et al. 2019, Wells and Clark 2019). Thus, evolutionary and ecological factors also interact to determine the success of host switching or host range expansion by parasites, making predictions of parasite community structure difficult.

With the absence of ecological and/or phylogenetic barriers, parasites are more likely to expand their host range resulting in zoonotic diseases, such as those spilling over into humans from rodent or bat reservoirs (Han et al. 2015, Morens et al. 2020) or from contact with domesticated animals (Woolhouse et al. 2001). Due to the potential fitness consequences of host switches to novel hosts (Liu et al. 2010, Kamath et al. 2019), it is important to understand the specificity with which parasite species infect their hosts and the contribution of host phylogenetic relatedness and environmental factors to the membership of parasite species in ecological communities. The ecological and evolutionary constraints of specialization result in a trade-off in which host generalists may achieve broader geographic distributions through the parasitism of many different host species (Medeiros et al. 2014), whereas host specialist parasites may achieve higher infection rates by maximizing resource acquisition and immune evasion within co-evolved host species (Longdon et al. 2014, Poulin and Morand 2014). Specialization, however, is not typically the result of co-speciation of parasites with their hosts. Indeed, we rarely see parasite phylogenies mirroring host phylogenies (Nieberding et al. 2010), suggesting that host switching—rather than co-speciation—is the dominant form of host–parasite evolution (De Vienne et al. 2013). Although sharing of host species is more likely to occur whether the novel host species is phylogenetically proximate to an existing host due to similarities in immune systems and resources available (Tinsley and Majorus 2007, De Vienne et al. 2009, Longdon et al. 2011, Clark and Clegg 2017), parasites are also able to jump into distantly related host species with high encounter rates due to shared geographic space. If so, ecological factors affecting host proximity may be more important in predicting parasite community structure, especially for host generalist parasites.

Still, it is challenging to tease apart the relative contribution of host breadth and environmental tolerances to parasite community structure because the presence of a host species in an ecological community is also largely a result of environmental tolerances (Sexton et al. 2009). A promising approach to separate the effects of host evolutionary history and ecological characteristics on parasite community structure is to focus on factors (e.g., relatedness and habitat characteristics of host species) that do not covary within the study system. For example, Locke et al. (2013) identified host phylogeny, rather than ecological characteristics, as the key predictor of metazoan parasite community similarity in six freshwater fish species by quantifying diet and habitat characteristics that did not mirror their phylogeny. In contrast, a study on 15 species of desert rodents found that host phylogeny was not a good predictor of ectoparasite, intracellular parasite, or helminth beta turnover in comparison with host environment characteristics such as host range size and trophic status (Dallas and Presley 2014). By focusing on ecological factors that do not covary with host phylogeny,
these studies were able to separate the effects of host evolutionary history and ecological characteristics on parasite community structure. The contradictory conclusions from these studies demonstrate the lack of universality among taxonomic groups in the importance of these niche characteristics. As parasite groups often do not share an evolutionary history, it is important to characterize the great diversity of host–parasite relationships across the tree of life.

The elevational distribution and phylogenetic relationships of Catharus thrushes (family Turdidae) in eastern North America present a unique opportunity to tease apart the relative contributions of host relatedness (a proxy for host immunological similarity) and elevation (a proxy for extrinsic environmental factors) on the degree of parasite sharing. The two most closely related host species in this clade, the Bicknell’s Thrush (Catharus bicknelli), and Veery (Catharus fuscens), breed at opposite ends of the sampled elevational gradients, while the Swainson’s Thrush (Catharus ustulatus) and Hermit Thrush (Catharus guttatus) have broad elevational ranges (Fig. 1; Able and Noon 1976). Haemosporidian parasites (order Haemospororida) have been studied in Catharus thrushes across large geographic scales and through their circannual cycle (Dodge et al. 2013, Pulgarín-R et al. 2019; Starkloff et al., 2020); however, little is known about the variability in infection among host species. The diversity of this parasite group allows for a thorough exploration of the variation in host sharing and specialization. Parasites in the genus Plasmodium (transmitted by Culicidae mosquitoes and traditionally referred to as “malaria parasites”) tend to be host generalists (Valkiunas 2004). Contrastingly, Leucocytozoon parasites (transmitted by Simulidae blackflies) and Haemoproteus parasites (subgenus Parahaemoproteus transmitted by Ceratopogonidae midges) tend to infect a single host species or genus (Valkiunas 2004, Moens and Pérez-Tris 2016, Galen et al. 2018). However, haemosporidian host specificity can change over evolutionary time (Galen et al. 2019) and across environmental gradients (Fecchio et al. 2019), and this may affect variation at the community level (García-Longoria et al. 2019).

First, we tested alternative hypotheses regarding parasite variation among species and across elevational zones using community similarity (or beta diversity), which can refer to the variation in the identity of community members or can also account for the phylogenetic relationship among community members (Webb 2000). As closely related host species tend to have more similar immune systems and resources for exploitation by parasites, we expected more closely related host species to harbor more similar parasite communities than less closely related host species. This effect should be stronger when looking at parasite groups that tend to have a smaller host breadth (e.g., Leucocytozoon and Haemoproteus) than groups that tend to have more generalized host relationships (e.g., Plasmodium). Alternatively, if host specialization is less important to infection than environmental tolerance, parasite communities are likely to be more variable across elevational zones than within the same elevational zone. If both independent variables are equally (un)important to parasite community structuring in thrushes, we are unlikely to see either of the above patterns.

Second, we document parasite lineage alpha diversity and infection prevalence to evaluate parasite patterns within species and elevational zones. Prevalence is commonly used as a measure of parasite infection rates across host populations or communities and is essential to disease ecology as it provides an indication of the relative success of a parasite species in infecting a population or community of hosts (Krasnov and Poulin 2010), but does not provide any indication of the fitness consequences the parasite may have on its hosts (Palinauskas et al. 2018). Although ecodegographic patterns of free-living organisms are well understood, large-scale patterns of parasite biodiversity are still being established (Preisser 2019). Based on previous findings of latitudinal and elevation gradients of haemosporidian parasites (Merino et al. 2008, Zamora-Vilchis et al. 2012, Cuevas et al. 2020, Fecchio et al. 2020), we expect the prevalence and diversity of Leucocytozoon to increase, and of Plasmodium and Haemoproteus to decrease, with increasing elevation.

**Materials and Methods**

**Host sampling**

Adult birds were captured in mist nets along established hiking trails or near roads in four...
mountain ranges in the Appalachian Highlands: the Catskill, Green, Adirondack, and White Mountains (Fig. 1). All samples were collected during the breeding seasons of 1993–2018 (late-May to early-July), in the form of blood or frozen tissue samples (Appendix S2). Blood samples were obtained using targeted mist netting and brachial venipuncture from banded and released birds. The tissue samples are associated with voucher specimens collected by researchers at the Cleveland Museum of Natural History, American Museum of Natural History, and New York State Museum. Previous studies conclude that blood samples and tissue samples have similar detectability of haemosporidian parasites (Svensson et al. 2007, Ramey et al. 2013, Pulgarín-R et al. 2019). Birds were sampled opportunistically, and samples were categorized into either high or low elevational zones based on the natural gap in our sampling that corresponds to the locality of the ecotonal change from deciduous to boreal forest in each mountain range (Fig. 1). Ecotones occur at lower elevations with increasing latitude along the Appalachian highlands (Cogbill and White 1991), precluding use of a single elevational threshold throughout our study region.

Samples were also collected from juvenile birds of three host species in the Green Mountains, Vermont, July: Bicknell’s Thrush (n = 76, collected in 2000–2002), Hermit Thrush (n = 7, collected in 2003–2004), and Swainson’s Thrush (n = 3, collected in 2004). These samples were only used to identify haemosporidian lineages that were locally transmitted to birds before their initial migration and were not included in any analyses. The majority of the juvenile Bicknell’s Thrush samples (n = 74) were collected from nestlings as described in Goetz et al. (2003). The remaining juvenile birds were fledged (but still adorned juvenile plumage) and sampled using the same methods as for adults. All applicable institutional and/or national guidelines for the care and use of animals were followed.
**Parasite sampling**

We extracted genomic DNA from all blood and tissue samples using a DNeasy Blood and Tissue extraction kit (QIAGEN, Valencia, CA) and targeted a 478 base pair fragment of mitochondrial gene cytochrome-b (cyt-b) using nested PCR (Hellgren et al. 2004). All negative PCRs (indicated by the absence of bands on 1.5% agarose gels) were repeated twice (for a total of three times) to establish that they were not false negatives. We cleaned positive amplicons using Exosap (ExoSAP-IT; Amersham Biosciences, Arlington Heights, Illinois, USA) and Sanger-sequenced amplicons on an ABI3700. We aligned sequences and edited them using Geneious Prime 2019.1.3. (https://www.geneious.com). We identified lineages by BLASTing to known sequences from MalAvi (Bensch et al.) and GenBank (Clark et al. 2016). A single, unambiguous nucleotide difference between sequences denoted different, potentially reproductively isolated lineages (Bensch et al. 2004, Pérez-Tris and Bensch 2005), and we treated each unique lineage as a separate unit of diversity for our analyses. We submitted novel lineages that did not match previously identified lineages to MalAvi (CAT-GUT04-06, CATUST40-42).

**Analyses**

We examined the effects of host relatedness and elevation on parasite community turnover (compositional and phylogenetic) within each haemosporidian genus and in all three genera combined. Linear regression models (lm function in the stats R package [R Core Team 2018]) were used to evaluate the variation in both turnover metrics among host species pairs and between elevational zones. In host models, host species pairs were assigned into three different categories of host divergence based on the Everson et al. (2019) phylogeny (Fig. 1): (1) Bicknell’s Thrush and Veery are the most closely related pair, (2) followed by their comparisons to the Swainson’s Thrush, and (3) lastly, all comparisons to the most distantly related Hermit Thrush. In elevation models, comparisons were made between high and low elevational zones (high–low) within each mountain range, and among high (high–high) and among low (low–low) elevational zones across mountain ranges. Individuals of any species were pooled into high or low elevational zones in each mountain range. We quantified beta diversity (compositional) and phylodiversity (phylogenetic) to evaluate dissimilarity among parasite communities using community matrices of parasite lineages and their abundances in each host species and elevational zones in each mountain range. We calculated beta diversity (chao dissimilarity index) among pairs of host species and between elevational zones in each mountain range using the vegdist function with chao index in vegan R package (Oksanen et al. 2013). Similarly, we calculated phylodiversity (mean pairwise distance) using the comdist function in picante R package (Kembel et al. 2010). This function incorporated the branch lengths from a maximum likelihood (ML) General Time Reversible (GTR) Gamma distributed tree with a proportion of invariant sites (GTR+G+I, 1000 replicate bootstrap) based on parasite mitochondrial cyt-b sequences constructed in MEGA7 (Kumar et al. 2016). It was rooted at the Leucocytozoon clade based on Borner et al. (2016).

We tested the effects of host species and elevational zone on alpha diversity using a linear regression model. We estimated alpha diversity (Shannon diversity index) in each host species and for each elevational zone from each mountain range. This diversity metric was calculated using rarefaction in the R package iNext (Hsieh et al. 2016), accounting for community abundance and evenness of lineages per genus (Spellerberg and Fedor 2003).

Lastly, to evaluate the effects of elevational zone and host species on the parasite prevalence (host infected or uninfected), we used a binomial generalized linear mixed-effects model (GLMM, glmer function in lme4 R package [Bates et al. 2007], logit link function). Elevational zone and host species were included as fixed effects in the model, with mountain range and a time variable as random effects. For this temporal variable, we partitioned sampling years into half decadal categories (e.g., 2001–2005 and 2006–2010), to account for non-continuous sampling. For each parasite genus, we ran models of varying combinations of predictive variables (see Table 1) and used ΔAICc to find the best model for predicting the infection status of birds (Burnham and Anderson 2004). For all analyses in R, we used version 3.5.2.
Table 1. Comparison of binomial GLMM models predicting the prevalence of Leucocytozoon and Plasmodium parasites in four closely related species of Catharus thrushes (n = 414).

| Parasite genus | Model | \( \text{AIC}_c \) | \( \Delta \text{AIC}_c \) |
|---------------|-------|-----------------|-----------------|
| Leucocytozoon | Host species + elevation | 327.4651 | 0 |
|               | Host species x elevation | 329.4216 | 1.9565 |
|               | Host species | 330.5176 | 3.0525 |
|               | Elevation | 331.4047 | 3.9396 |
|               | Random effect only | 335.6027 | 8.1376 |
| Plasmodium | Host species | 464.5979 | 0 |
|              | Host species + elevation | 466.4389 | 1.841 |
|              | Host species x elevation | 467.0612 | 2.4633 |
|              | Random effects only | 476.5932 | 11.9953 |
|              | Elevation | 477.8204 | 13.2045 |

Notes: Models are ranked in ascending order of \( \Delta \text{AIC}_c \), relative to the model with the lowest \( \text{AIC}_c \) for that parasite genus (bolded). All models included the temporal variable (half decadal category) and site as random effects in addition to fixed variables listed. We were unable to model Haemoproteus infection status due to the rarity of infection (2.4% infected).

Results

We identified a total of 38 haemosporidian lineages (Fig. 2), infecting 366 of the 414 birds sampled (88.4%; 25.1% coinfected by parasites in two genera). These infections were caused primarily by parasites in the genus Leucocytozoon (85.3% infection rate, 23 lineages). Plasmodium parasites comprised 10 lineages (26.8% of infections). Haemoproteus parasites (5 lineages) were rare (2.4%) and so were excluded from individual genus statistical comparisons. We identified six new Leucocytozoon lineages, all occurring as singleton infections (Fig. 2). While rare lineages (~2% prevalence) were typically found as singletons in species within the three closely related host species, most of the more common lineages (n = 8) infected all four host species at similar prevalence. We identified four lineages in the juvenile birds, confirming local transmission on the breeding grounds: P_CATUST05 (n = 1), P_BT7 (n = 1), L_CATUST11 (n = 3), and L_CATMIN07 (n = 3).

Beta diversity analyses indicated a trend of higher chao dissimilarity and higher mean pairwise distance of Leucocytozoon communities with increasing host genetic divergence, whereas Plasmodium communities did not significantly vary in either beta diversity measure with host divergence (Fig. 3, left panel). Leucocytozoon and Plasmodium communities did not have higher dissimilarity between low and high elevations within a mountain range than in high or low elevations across mountain ranges (Fig. 3, right panel). However, Plasmodium communities were most dissimilar at high elevations across mountain ranges, though this effect was less pronounced when looking at parasite phylogenetic dissimilarity (Fig. 3, right panel). When including all three genera, both beta diversity measures largely mirrored patterns of Leucocytozoon (Appendix S1: Fig. S1). Alpha diversity of Plasmodium and Leucocytozoon parasites did not differ significantly among host species or between elevational zones (Appendix S1: Fig. S2). However, thrushes in this study system (across all four mountains) were infected by a more diverse assemblage of Leucocytozoon parasites than Plasmodium parasites (Fig. 2).

The best prevalence model for Leucocytozoon contained host species and elevational zone without an interaction term (Table 1). The high level of variability suggests that Catharus tends to have high Leucocytozoon infection prevalence regardless of species, though prevalence was typically lower at high elevation (mean = 0.754; 95% CI lower = 0.512, upper = 0.899) than low elevation (mean = 0.948; 95% CI lower = 0.858, upper = 0.982; Fig. 4). The best model for Plasmodium prevalence was the host species only model (Table 1), with the lowest prevalence in the closely related Bicknell’s Thrush (mean = 0.125; 95% CI lower = 0.074, upper = 0.203) and Veery (mean = 0.240; 95% CI lower = 0.167, upper = 0.333), higher in the Swainson’s Thrush (mean = 0.297; 95% CI lower = 0.208, upper = 0.405), and highest in the most distant host species, the Hermit Thrush (mean = 0.449; 95% CI lower = 0.346, upper = 0.557; Fig. 4).

Discussion

A haemosporidian parasite community is impacted by each lineage’s ability to infect different host species and a wide variety of abiotic and biotic factors (Poulin and Krasnov 2010, Sehgal et al. 2011, Fecchio et al. 2019, Wells and Clark...
We used a model avian host system of temperate forest-breeding thrushes in the Appalachian Highlands to tease apart the effects of host relatedness and elevation on haemosporidian community structure. *Leucocytozoon* parasites seem to show host delineation, leading to more similar parasite communities in more closely related host species despite the separation of hosts along elevational gradients. Contrastingly, *Plasmodium* communities were equally dissimilar among all host species comparisons, suggesting that the sharing of lineages is not determined by host relatedness.
Leucocytozoon parasites are typically more prevalent and abundant in colder, temperate climates, as are their black fly vectors (Haas et al. 2012, McCreadie and Adler 2014, Oakgrove et al. 2014, McCreadie et al. 2018, Cuevas et al. 2020, Fecchio et al. 2020). The temperate, forested breeding habitats of Catharus thrushes provide ideal conditions for high Leucocytozoon infection rates across their geographic ranges (Galen et al. 2018; Starkloff et al., 2020). Consistent with these
patterns, we found the *Catharus* thrushes in this study infected at a very high prevalence by *Leucocytozoon* parasites (88.4% on average). We also found a high diversity of *Leucocytozoon* lineages across the host species and sites sampled including a total of 23 lineages, six of which have not been discovered before. Optimal climate conditions in northern latitudes likely provide increased resource stability for *Leucocytozoon* parasites and their vectors, which may lead to higher rates of competition among individual lineages (Futuyma and Moreno 1988). While we observed increased dissimilarity of *Leucocytozoon* parasite communities with decreased host relatedness, overall beta turnover of *Leucocytozoon* was low (mean chao dissimilarity = 0.19) as these communities were overrepresented by the highly prevalent L_CATUST11 (>25%) and L_CATMIN07 (10–25%) in all host species (Fig. 2). The classic trade-off hypothesis states that a specialist parasite achieves higher prevalence within its co-evolved host and generalist parasites infect a greater diversity of host species at lower prevalence (Medeiros et al. 2014). However, our findings provide evidence for the resource-breadth hypothesis which states that the breadth of hosts reflects the parasite species’ host use proficiency resulting in higher prevalence by parasites with larger host breadths (Medeiros et al. 2014). Indeed, L_CATUST11 and L_CATMIN07 infect all four host species with high prevalence. These two lineages may be in competition with one another as we see a fluctuation in their dominance at larger spatial scales, where L_CATMIN07 is more prevalent than L_CATUST11 further south of our study system (Starkloff et al., 2020).

The differences that we see among host pairs in *Leucocytozoon* community turnover are a result of variability in parasite sharing of rarer lineages that are typically absent in the Hermit Thrush. For example, L_POEHUD01 (only reported in *Catharus minimus*, the sister species to the Bicknell’s Thrush, in MalAvi; Bensch et al. ) is present in the Swainson’s Thrush (<2% prevalence) and is often detected (2–10%) in the closely related Bicknell’s Thrush and Veery (Fig. 2), but was absent in the Hermit Thrush (Fig. 2, Bensch et al. 2009).
two closest relatives of L_POEHUD01 are also absent from the Hermit Thrush in our study (Fig. 2), suggesting that this clade of parasites may not be able to infect the more distantly related species despite being transmitted in the same low elevation habitats. The absence of this parasite clade in the Hermit Thrush, which is more closely related to non-migratory, neotropical Catharus thrushes than to the other migratory Catharus thrushes (Everson et al. 2019), may be a result of considerable differentiation in molecular pathways limiting successful infections by more specialized parasites (Longdon et al. 2014). Future work should examine molecular pathways crucial to host cell invasion and parasite evasion to determine the genetic underpinnings of parasite sharing among hosts of differing relatedness. When combining all three haemosporidian genera, community turnover patterns largely mirror those of Leucocytozoon, demonstrating the importance of looking at a smaller taxonomic scale to elucidate genus-level patterns. Plasmodium parasite communities are not determined by host relatedness, or (like Leucocytozoon) by elevation. Plasmodium community composition was, however, most heterogeneous among high elevation zones of the four mountain ranges, suggesting a sky-island effect (Williamson et al. 2019). Plasmodium parasites and their mosquito vectors are less abundant and diverse in colder climates (Foley et al. 2007, Merino et al. 2008), which could limit their transmission between the tops of mountain ranges. Plasmodium parasite communities were substantially less diverse and more genetically divergent in our study system than Leucocytozoon parasite communities which are comprised of many closely related parasites. Thus, the addition or removal of a single Plasmodium lineage can have a larger effect on community turnover measures than a Leucocytozoon lineage. Patterns of substantially higher turnover of Plasmodium communities than Leucocytozoon communities are also seen over a larger spatial scale in Catharus thrushes (Starkloff et al., 2020).

Plasmodium communities have high dissimilarity across all species comparisons (mean chao dissimilarity = 0.63). As all four host species winter allopatrically in Central and South America (Clement 2000), we must consider the possibility that Plasmodium infections could have been acquired on the wintering grounds or during migratory stopovers (Waldenström et al. 2002, Altizer et al. 2011). However, Soares et al. (2019) found that neotropical migrants sampled on their wintering grounds have parasite assemblages that are distinct from those of resident species, suggesting little to no transmission between these two groups of seasonally co-occurring birds. Within the Catharus study system, Pulgarin-R et al. (2019) showed that the sister species of the Bicknell’s Thrush, the migratory Gray-cheeked Thrush (C. minimus), showed limited potential as a long-distance disperser of haemosporidian parasites. Additionally, the four lineages (P_CATUST05, P_BT7, L_CATMIN07, L_CATUST11) that we found in juvenile birds sampled prior to their first migration confirms their local transmission on the breeding grounds. The latter three locally transmitted lineages have previously been documented in juvenile birds in western North America, suggesting widespread transmission across the continent (Dodge et al. 2013, Cozzarolo et al. 2018, Pulgarin-R et al. 2019). The comparable similarity of Leucocytozoon communities both within and between closely related Catharus species (Starkloff et al., 2020) suggests that Leucocytozoon transmission outside of their temperate breeding grounds is improbable and unlikely to impact parasite community structure.

The prevalence of Plasmodium varies considerably among the four host species, with the highest prevalence in the Hermit Thrush. High variability in Plasmodium community structure among all species pairs may, thus, be driven more so by large disparities among host species in infection prevalence of lineages than the presence/absence of lineages (Fig. 2). High Plasmodium prevalence in the Hermit Thrush may be a result of higher host susceptibility or increased host tolerance of the infection (Miller et al. 2006, Råberg et al. 2009), or higher transmission rates achieved in a host species typically found at high density (Arneberg et al. 1998). The Hermit Thrush occupies the largest elevational breadth (Able and Noon 1976) and the broadest range of habitat types (Dellinger et al. 2012) of our host species, likely exposing it to a wider variety of parasitic organisms (Johnson et al. 2016). As a result, a specialized immune response may be less beneficial than tolerance mechanisms that may lower the fitness costs of infection for species harboring
diverse assemblages of parasites. Contrastingly, *Leucocytozoon* prevalence was similar among the four host species. We did, however, find a slight decrease with elevation, despite the tendency of *Leucocytozoon* prevalence to be higher in colder conditions (Haas et al. 2012, Oakgrove et al. 2014, Cuevas et al. 2020, Fecchio et al. 2020). Haemosporidian communities in *Catharus* thrushes are clearly not structured by thermal tolerances alone, and the elevational decrease in *Leucocytozoon* prevalence is likely the result of some other factor associated with high elevation habitat, such as smaller streams that are typical of high elevations harboring a lower abundance of black fly larvae than larger streams and rivers (Malmqvist 2002). Alternatively, the host breadth of haemosporidian lineages may have decreased as climatic conditions change with elevation (Fecchio et al. 2019), thus, decreasing the probability of lineages infecting birds at high elevations. While this is unlikely at our spatial scale, our four host species study system is not suitable to evaluate whether host breadth of parasites varied with elevational climatic differences. Screening all avian host species across these elevational gradients would provide a better understanding of how the host breadth of a parasite lineage varies across its range.

As host breadth is typically variable across much larger spatial scales (Fecchio et al. 2019), the regional scale of our study was informative in revealing the disparate importance of host relatedness in structuring communities of two haemosporidian genera sampled across an environmental gradient. The collaborative archiving of avian haemosporidian parasites and their known avian host associations (Bensch et al.) makes these parasites an ideal taxonomic group to test whether these patterns are consistent in different climatic conditions and host communities in the future.

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