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HAEMOSPORIDIANS AS POTENTIAL SOURCES OF GEOGRAPHIC MARKERS FOR IRRUPTIVE BLACK-CAPPED CHICKADEES

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ABSTRACT

Migratory birds can carry parasites thousands of miles, and therefore their parasite communities may provide an informative means of ascertaining the breeding and wintering locations of their hosts. However, avian parasites (particularly haemosporidians) are rarely used for this purpose because their usefulness is conditional upon knowing where and when birds acquired them during their annual cycles. In this study, we evaluated haemosporidian assemblages among non-resident black-capped chickadees (*Poecile atricapillus*), which occasionally "irrupt" south from parts of their northern distributional range to spend winters in the mid-Atlantic and Midwest regions of the United States. Specifically, we compared haemosporidian prevalence and lineage diversity from irruptive black-capped chickadees to those of resident Carolina chickadees (*Poecile carolinensis*) and black-capped × Carolina hybrids at 4 southeastern Pennsylvania locations, where most irruptive black-capped chickadees could be genetically and phenotypically identified among local birds. We hoped to (1) gauge whether certain haemosporidian lineages could help identify irruptive individuals among populations of otherwise sedentary black-capped chickadees and (2) roughly estimate where these irruptive birds hatched or bred. Irruptive birds had higher rates of haemosporidian infection than residents, and irruptive birds exclusively harbored a single *Plasmodium* lineage (BT7). Literature review suggests that lineage BT7 is widespread across the Northern hemisphere but is unlikely to be transmitted between chickadees in our study location. Thus, the presence of lineages such as BT7 may be useful for assessing the composition of seasonally sympatric bird populations with different migratory strategies or at least for identifying non-resident individuals within these populations.

Avian haemosporidians are intracellular blood parasites that have long been studied as models for infectious diseases (Rivero and Gandon, 2018) in the context of host-parasite coevolution (Ricklefs et al., 2014; Alcala et al., 2017) and as sources of mortality in wild bird populations (van Riper et al., 1986; Gulliver et al., 2022). Three haemosporidian genera infect birds, and each genus is transmitted by a different suite of insect vectors: *Plasmodium* by mosquitoes (Culicidae), *Leucocytozoon* by blackflies (Simuliidae), and *Haemoproteus,* which includes the subgenera *Haemoproteus* and *Parahaemoproteus,* by louse flies (Hippoboscidae) or biting midges (Ceratopogonidae), respectively (Valkiūnas, 2005).

With the increasing availability of molecular tools for detecting parasites, the number of published studies on avian haemosporidians has grown exponentially in the last 2 decades (Bensch et al., 2009; Marzal, 2012; Rivero and Gandon, 2018). However, haemosporidian research remains riddled with challenges and knowledge gaps because live parasites cannot be directly observed in situ, their life histories make them difficult experimental subjects, and their species delimitations are largely unresolved (Outlaw and Ricklefs, 2014). One knowledge gap concerns a basic understanding of haemosporidian biogeography, which is complicated because haemosporidian hosts are long-distance migrants that can transport these parasites across geographic barriers and connect parasite communities that would otherwise remain isolated (de Angeli Dutra et al., 2021). As the most mobile group of terrestrial vertebrates, birds and their migratory habits can therefore complicate our understanding of where certain haemosporidians originated and evolved, and how they are transmitted by insect vectors.

Avian migration probably aids in long-distance haemosporidian dispersal, given that many migratory birds acquire infections on their breeding grounds (Pérez-Tris and Bensch, 2005; Cozzarolo et al., 2018; Pulgarín-R et al., 2019) and in tropical wintering regions (Waldenström et al., 2002; Hasselquist et al., 2007; Jones et al., 2018).

However, the paucity of suitable vectors in certain areas (Hellgren et al., 2007; Pulgarín-R et al., 2019) and the phenological mismatch of birds' presence in these regions with seasonal vector activity (Loaiza and Miller, 2013; Hernández-Lara et al., 2017) may act as barriers that reduce or preclude local parasite transmission. Likewise, physiological or immunological factors within hosts (Soares et al., 2020) that decrease parasitemia and transmissibility may also prevent many haemosporidian lineages from dispersing across continents.

If ecological barriers restrict haemosporidian transmission to certain regions, some haemosporidian lineages could serve as geographic markers to track birds' seasonal movements. Several researchers have proposed screening birds for regionally transmitted parasites as a supplemental or alternative approach to band/recapture methods, stable isotope analyses, or satellite telemetry studies, which are typically used to deduce where birds migrate (Rintamäki et al., 1998; Webster et al., 2002; Shurulinkov and Ilieva, 2009; Marzal, 2012). A few authors have even tested the utility of "haemosporidian markers," though the effectiveness of such markers seems to vary by system. For instance, haemosporidian communities among breeding black-throated blue warblers (*Setophaga caerulescens*) (Fallon et al., 2006) and common yellowthroats (*Geothlypis trichas*) (Pagenkopp et al., 2008) provide little geographic signal, possibly due to natal dispersal of hosts between breeding populations, parasites being widespread host generalists, or a combination of both factors. However, Durrant et al. (2008) found regional differences in haemosporidian communities across the breeding range of American redstarts (*Setophaga ruticilla*), and for European barn swallows (*Hirundo rustica*) *Plasmodium* prevalence and lineage composition were predicted by birds' overwintering locations in Africa (von Rönn et al., 2015). Moreover, several lineages in the swallows were decidedly obtained in Africa, having never been recorded in resident or juvenile European birds. Furthermore, Galen et al. (2024) found that haemosporidian communities differ among stopover migrant birds and resident birds at a site in southeastern (SE) Pennsylvania.

To make the most use of haemosporidians as geographic markers, there must be some certainty regarding where their hosts acquired their parasites (breeding grounds, wintering areas, stopover sites, etc.). In some cases (as with von Rönn et al., 2015), this certainty can be achieved through careful analysis of haemosporidian records in other seasonally sympatric hosts, especially when those hosts are nonmigratory. This approach may be problematic for novel parasite lineages or those with few host records to begin with. For birds that winter in areas too cold or dry to support year-round vector populations, their breeding grounds would be the only source of infections, eliminating any confusion about where haemosporidian transmission occurred. However, no studies thus far have focused on haemosporidian markers for birds that exclusively occur in seasonally cold, temperate climates.

In North America, at least 2 dozen passerine and raptor species that breed in boreal regions exhibit sporadic southbound movements (known as irruptions) triggered by productive breeding and high population densities followed by autumn food shortages (Hussell and Stamp, 1965; Bock and Lepthien, 1976; Koenig, 2001). Included among these species are black-capped chickadees (*Poecile atricapillus*; BCCH). Although most BCCH are sedentary (Brewer et al., 2000; Foote et al., 2020) and many breed south of boreal habitats, BCCH from the northern parts of their range (e.g., Quebec and Ontario) may "irrupt" in large numbers when resources are scarce and cannot support BCCH winter presence in these regions (Bock and Lepthien, 1976; Koenig and Knops, 2001).

In the northeastern United States, irruptive BCCH often arrive in October and return north the following spring (Foote et al., 2020; Mostrom et al., 2020). Where the more southerly-distributed Carolina chickadee occurs (*Poecile carolinensis*; CACH), invading BCCH integrate into CACH flocks and retreat before the onset of the CACH breeding season (Simon, 1956, 1959; Merritt, 1981). However, little else is known about BCCH irruptions, and the tendency for irruptive chickadees to associate with local conspecifics in more northerly regions makes their movements difficult to track and quantify. Moreover, these periodic influxes of BCCH can confound work that is otherwise focused on sedentary chickadee populations.

In this study, we tested haemosporidians as markers for identifying irruptive BCCH in SE Pennsylvania and roughly determining the origins of these birds. In Pennsylvania, resident BCCH reach their regional southern range limit, defined by a narrow hybrid zone with CACH (Reudink et al., 2007). In this region, the only times when BCCH occur south of this hybrid zone are in fall and winter, where they join CACH flocks (Mostrom et al., 2020) and are visually distinguishable from the darker-winged, shorter-tailed CACH (Pyle, 1997; Bolgiano, 2004).

Here, we compared haemosporidian prevalence and diversity among irruptive BCCH with that of resident BCCH, CACH, and BCCH × CACH hybrids screened from our study spanning the BCCH × CACH hybrid zone (Rice et al., 2021). Specifically, we asked (1) whether haemosporidian prevalence differs between resident and irruptive chickadee hosts; (2) whether irruptive BCCH are infected by unique haemosporidian lineages that set these birds apart from resident chickadees; and (3) whether such haemosporidian lineages could serve as markers for identifying the geographic origins of irruptive BCCH. We predicted substantial variation between local (sedentary) and non-local (irruptive) chickadee haemosporidian communities because chickadees are (1) largely sedentary outside of irruption events (Foote et al., 2020), (2) likely exposed to different vectors and climatic factors on their respective breeding grounds, and (3) unlikely to exchange parasites during the October–March interval when irruptions occur.

MATERIALS AND METHODS

Field and lab protocols

Following Rice et al. (2021), we collected chickadee blood samples from 4 long-term study sites spanning the BCCH × CACH hybrid zone in SE Pennsylvania (Fig. 1). We specifically targeted irruptive BCCH during the winters of 2012, 2016, and 2018 and sampled resident chickadees from a broader time frame during the 2006–2019 breeding seasons (May–June) and nonbreeding/overwintering periods (October–March 2012–2019). The 2 northernmost sites were Tuscarora State Park (TU) (40°48′19.43″N, 76°1′29.28″W) and Hawk Mountain Sanctuary (HM) (40°38′2.37″N, 75°59′13.79″W), which both have resident BCCH. The 2 southern sites were the Nolde Forest Environmental Education Center (NF) (40°16′12.01″N, 75°56′59.81″W) and Great Marsh (GM) (40°8′24.07″N, 75°43′48.31″W), where

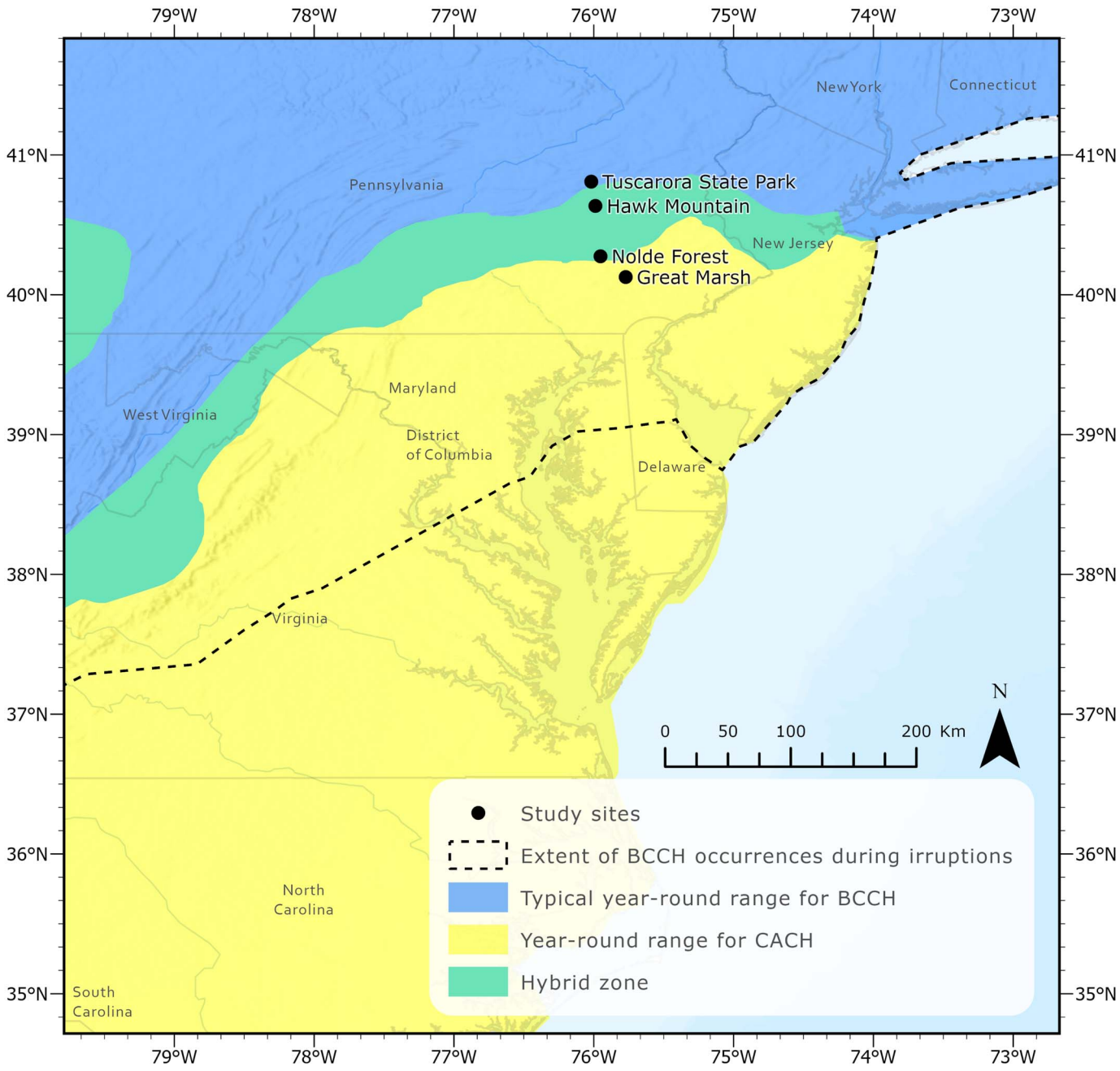

Figure 1. Map of the *Poecile atricapillus* (black-capped chickadee; BCCH) × *Poecile carolinensis* (Carolina chickadee; CACH) hybrid zone and extent of BCCH irruptions within the mid-Atlantic region of the United States, based on eBird observations inputted into ArcGIS Pro 3.2.1. The area between the southern edge of the hybrid zone and the dotted line is based on the discrepancy of BCCH observations between an October–March window (when irruption events occur) and an April–September window. Study sites are depicted as dots straddling the hybrid zone in southeastern Pennsylvania. GM = Great Marsh, HM = Hawk Mountain, NF = Nolde Forest, and TU = Tuscarora State Park. Color version available online.

all resident chickadees are CACH or CACH-like backcrosses (Taylor et al., 2014a; Driver et al., 2022), and BCCH are present only during major irruptive events.

During irruption events, BCCH temporarily appeared at GM and NF, and the proportions of BCCH at HM increased. We captured and processed these BCCH in the same manner as used for residents, by placing mist nets near feeders. We aged birds based on tail feather shape and wear (Pyle, 1997) and obtained blood samples (~30 µl) by brachial venipuncture. We then stored blood samples in ~300 µl of Queen's lysis buffer (Seutin et al., 1991) at 4 C.

We extracted DNA from buffered blood samples with the DNeasy Blood and Tissue Kit (Qiagen, Germantown, Maryland) following the manufacturer's protocol, although some earlier extractions were conducted with a modified version (Driver, 2017) of the Puregene Core A extraction kit (Qiagen). We then screened extractions for haemosporidian DNA by amplifying cyt *b* fragments by using a nested PCR protocol (described by

Rice et al., 2021). We sent positive PCR products to Functional Biosciences (Madison, Wisconsin) for Sanger sequencing, trimmed and reconciled sequences in Geneious v8.1.8 (Kearse et al., 2012), and identified consensus sequences as specific haemosporidian lineages by performing BLAST searches of their sequences with the MalAvi database (Bensch et al., 2009). We numbered clean sequences that did not match previously-named lineages in MalAvi with the prefix POEATR. Infections with multiple haemosporidian lineages were identified via cyt *b* chromatograms in Geneious following the methodology of Galen et al. (2019). Here, a chromatogram with multiple peaks in 1 or more nucleotide positions was indicative of a mixed infection, and sets of peaks that differed consistently in height were manually phased before performing BLAST searches. All haemosporidian cyt *b* sequences from this study (including novel lineages) can be found on MalAvi and GenBank (accession numbers PV916661–PV916716).

Identifying irruptive chickadees

We identified chickadees as BCCH, CACH, and hybrids through a PCR-RFLP genotyping approach developed by McQuillan et al. (2017) and used by Rice et al. (2021) to assay 8 nuclear SNP markers with alleles that identify BCCH or CACH. We visualized the restriction enzyme-digested PCR products on gels and determined each bird's taxonomic identity based on the percentage of species-specific alleles it possessed.

Generally, we ruled out all birds that carried ≥1 CACH allele, bred locally, or remained present over multiple years as irruptive BCCH, although our means for further identifying irruptive BCCH differed by location. At GM, which lies south of the hybrid zone and harbors only CACH as a breeding species (Taylor et al., 2014b; Driver et al., 2022), we classified any bird with BCCH phenotypic characters as irruptive (e.g., proportionately long tail feathers and white-edged secondaries/secondary coverts). These BCCH phenotypes were diagnostic for all but 5 individuals, which we confirmed as BCCH with a single RFLP assay at the *cop238* locus. At NF, where unadmixed BCCH have not bred since the mid-2000s (Taylor et al., 2014a; Wagner et al., 2020), we identified irruptive birds by genotype (absence of CACH alleles). At HM and TU, we also genotyped birds that were captured during irruption events. However, because BCCH breed at these northerly sites (Driver et al., 2022), we could not confirm irruptive status for any overwintering BCCH at HM or TU. Thus, we omitted these possibly irruptive BCCH from our main analyses and address them separately in our results.

Statistical analyses

We used Pearson's chi-square tests with Yates's correction for continuity to compare overall haemosporidian prevalence, prevalence of each haemosporidian genus, and prevalence of specific haemosporidian cyt *b* lineages in resident vs. irruptive chickadees. Because Rice et al. (2021) found infection patterns that were similar across BCCH, CACH, and hybrids, we included in the present analyses residents from all 3 taxonomic groups. However, parasite prevalence was strongly seasonal, and because winter-sampled BCCH residents at TU and HM were indistinguishable from irruptive birds unless they were also detected at other times of year, we lacked sufficient sample sizes to make meaningful comparisons between these 2 categories of BCCH. Instead, we performed 2 separate sets of tests: 1 test that compared irruptive BCCH against all other winter-sampled chickadees (BCCH, CACH, and hybrids in 1 category) and a second test that compared irruptive birds against local BCCH that were sampled during the breeding season. Within these sets, we performed separate tests for each haemosporidian genus and lineage that infected ≥4 individual hosts (Suppl. Table S1).

We identified approximately 95% of irruptive BCCH as birds <1 year old, and because host age may predict haemosporidian prevalence (Sol et al., 2003; Carlson et al., 2018), we compared haemosporidian prevalence (by parasite genus) between all wintering adult and yearling chickadees (regardless of irruptive status) via Pearson's chi-square tests and repeated these analyses for irruptive vs. resident yearlings (Table S1). We used Cramér's V to measure effect size for all chi-square tests, and for each set of comparisons (e.g., irruptive birds vs. winter residents, or yearlings vs. adults), we accounted for multiple testing by applying a Benjamini-Hochberg procedure with a false discovery rate set to 5% (Benjamini and Hochberg, 1995) (Table S2). We conducted all statistical analyses using the *stats* and *confintr* (Mayer, 2025) packages in R v4.0.5 (R Core Team, 2021).

RESULTS

Overall findings

We screened 53 irruptive BCCH from GM and NF for haemosporidians, 28 (52.8%) of which were infected. We identified all but 1 instance of infection to parasite genus and obtained unambiguous cyt *b* sequences for all but 1 infection. The parasite genera included *Plasmodium, Leucocytozoon,* and *Haemoproteus* (subgenus *Parahaemoproteus*). Similar to resident chickadees (Rice et al., 2021), *Plasmodium* was the most prevalent genus in irruptive BCCH (n = 19), followed by *Leucocytozoon* (n = 8) and *Haemoproteus* (n = 4) (Table I). Five irruptive BCCH hosted mixed infections: 4 had a *Leucocytozoon*/*Plasmodium* combination and another had multiple *Haemoproteus* lineages. Lineage richness was lower in irruptive BCCH (8 lineages) than in resident BCCH (12 lineages) and winter-sampled chickadees (11 lineages). Two lineages in irruptive BCCH (belonging to *Leucocytozoon* and *Haemoproteus*) were novel in that they did not match previously recorded haemosporidian cyt *b* sequences found in the MalAvi database (Table I).

We screened an additional 46 BCCH from HM and TU that could not be confirmed as irruptive individuals (Table II). Of these, 23 (50%) harbored infections, and all infections were identified to parasite genus. Again, *Plasmodium* (n = 16) was the most common genus, followed by *Leucocytozoon* (n = 6) and *Haemoproteus* (n = 3). However, 5 infections comprised more than 1 parasite haplotype; 3 of these "mixed" infections contained haemosporidians that we could not identify to specific lineages.

Differences in parasite prevalence

Irruptive BCCH (with 52.8% infected) were more likely to carry detectable haemosporidian infections than were resident winter-sampled BCCH, CACH, and hybrids (29.6% infected; $\chi^2 = 8.8$, df = 1, $P = 0.003$, Cramér's V = 0.179) or resident BCCH sampled during breeding seasons (31.1% infected; $\chi^2 = 7.2$, df = 1, $P = 0.007$, Cramér's V = 0.195) (Fig. 2). *Plasmodium* infections

Table I. List of haemosporidian cyt *b* lineages detected in irruptive and resident chickadees from 4 sites in southeastern Pennsylvania. Lineages that could identify black-capped chickadees (BCCH) as non-resident/irruptive individuals are in bold. Novel lineages are in italic.

	No. of birds		
Lineage name*	Irruptive BCCH	All winter-resident chickadees†	Locally-breeding BCCH†
L_CARCAR09	0	0	1
L_CB1	7	11	3
L_COLBF21	0	4	4
L_DUMCAR02	0	0	3
L_SETCOR07	0	2	0
L_POEATR06	1	0	0
L_POEHYB03	0	1	0
P_BAEBIC04	2	32	25
P_BT7	**10**	**0**	**0**
P_CORPIL02	0	0	1
P_GEOTRI09	**6**	**0**	**1**
P_PADOM11	0	1	2
P_POEATR02	0	0	1
P_POEATR04	1	0	0
P_POEATR05	0	0	1
H_MAFUS02	3	17	0
H_POEATR07	1	1	0
H_POEATR08	0	0	1
H_POEATR09	0	0	1
H_POECAR03	0	1	0
H_POECAR04	0	1	0
H_POECAR05	0	1	0
Total no. of birds infected‡	28	77	55
Total no. of birds sampled	53	253	161

* L = *Leucocytozoon,* P = *Plasmodium,* H = *Haemoproteus.*
† Individuals screened by Rice et al. (2021).
‡ Includes low-quality sequences and samples containing multiple lineages.

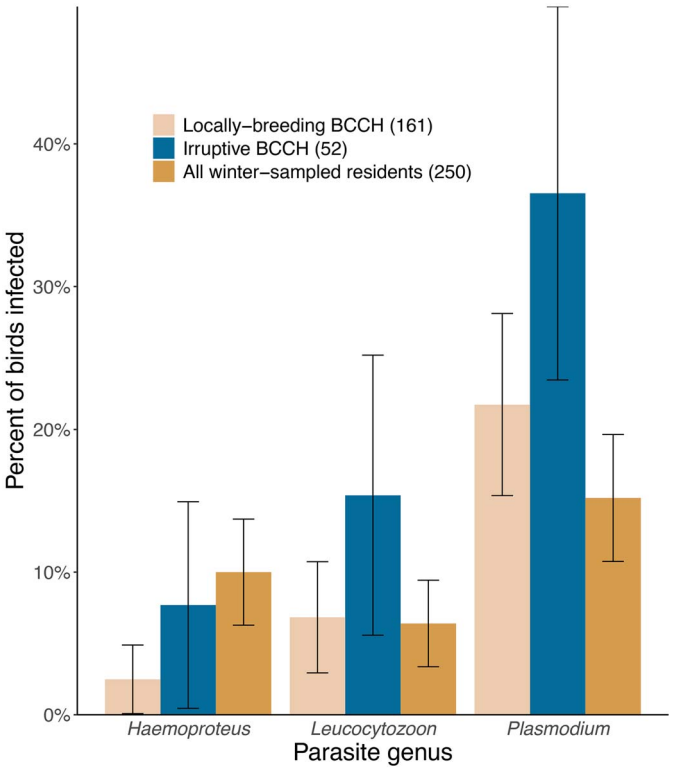

Figure 2. Prevalence of haemosporidians among irruptive *Poecile atricapillus* (black-capped chickadee; BCCH), winter-sampled residents, and locally-breeding BCCH (with 95% confidence intervals). *Leucocytozoon, Plasmodium,* and *Haemoproteus* infections were pooled across all sites. Numbers in parentheses indicate sample sizes. Color version available online.

were more than twice as prevalent in irruptive BCCH (36.5% of birds) than in winter residents (15.2%; $\chi^2 = 11.4$, df = 1, $P < 0.001$, Cramér's V = 0.206) and were more prevalent in irruptive than in locally-breeding BCCH (21.7%) but not significantly ($\chi^2 = 3.8$, df = 1, $P = 0.051$, Cramér's V = 0.146). *Leucocytozoon* infections occurred in 15.4% of irruptive BCCH but were not significantly less prevalent in winter residents (6.4%; $\chi^2 = 3.6$, df = 1, $P = 0.057$, Cramér's V = 0.125) or locally-breeding BCCH (6.8%; $\chi^2 = 2.6$, df = 1, $P = 0.109$, Cramér's V = 0.129). *Haemoproteus* occurred at similar frequencies in all 3 groups, infecting 7.7% of irruptive BCCH, 10% of winter residents ($\chi^2 = 0.1$, df = 1, $P = 0.799$, Cramér's V = 0.03), and 6.8% of locally-breeding BCCH ($\chi^2 = 1.7$, df = 1, $P = 0.194$, Cramér's V = 0.118).

Table II. Haemosporidian prevalence in black-capped chickadees (BCCH) with unknown origins. Lineages that could identify BCCH as non-resident/irruptive individuals are in bold.

Lineage name*	No. (%) of birds infected
L_CB1	5 (10.9)
L_COLBF21	1 (2.2)
P_BAEBIC04	7 (15.2)
P_BT7	**2 (4.3)**
P_GEOTRI09	**5 (10.9)**
P_PADOM11	1 (2.2)
P_POEATR03	1 (2.2)
H_MAFUS02	3 (6.5)
Total no. of birds infected†	23
Total no. of birds sampled	46

* L = *Leucocytozoon,* P = *Plasmodium,* H = *Haemoproteus.*
† Includes samples infected by multiple haemosporidian lineages.

CB1, which was the most common *Leucocytozoon* lineage in our study, was more prevalent among irruptive birds (13.5%) than among winter-sampled residents (4.4%; $\chi^2 = 4.8$, df = 1, $P = 0.029$, Cramér's V = 0.145) and locally-breeding BCCH (1.9%; $\chi^2 = 9.4$, df = 1, $P = 0.002$, Cramér's V = 0.236). However, 2 *Leucocytozoon* lineages (DUMCAR02 and COLBF21) that were common among breeding residents (Rice et al., 2021) were absent among irruptive BCCH. BAEBIC04, a *Plasmodium* lineage that accounts for half of all infections in resident chickadees, was rarer among irruptive BCCH (3.8%) than among BCCH residents sampled during the breeding season (15.5%; $\chi^2 = 3.9$, df = 1, $P = 0.048$, Cramér's V = 0.151), but this trend was not significant after correcting for multiple testing (Table S2). Two *Plasmodium* lineages (BT7 and POEATR04) and 1 *Leucocytozoon* lineage (POEATR06) occurred in only irruptive BCCH, although POEATR04 and POEATR06 were single occurrences. Another *Plasmodium* lineage (GEOTRI09) was exclusive to irruptive birds apart from a single infected BCCH that bred at HM (Table I). Still, lineages BT7 and GEOTRI09 infected 16 of 52 irruptive BCCH (Table I), and their (near) absence in resident chickadees during winter ($\chi^2 = 43.9$, df = 1, $P < 0.001$, Cramér's

V = 0.406 for BT7; $\chi^2 = 23.8$, df = 1, $P < 0.001$, Cramér's V = 0.312 for GEOTRI09) suggests that these parasites are widely transmitted where these birds breed but less so or not at all within our SE Pennsylvania study sites. Among 46 winter-sampled BCCH that we could not identify as either migrants or residents, 7 instances of infection with lineages BT7 and GEOTRI09 were found (Table II). Lineages BT7 and GEOTRI09 were found in all 3 irruption years (2012, 2016, and 2018) but were most numerous in 2012.

Infections as a function of host age

Host age was minimally correlated with haemosporidian prevalence. We successfully aged all but 4 winter-captured birds (2 irruptive BCCH and 2 resident CACH) as yearlings (n = 136) or adults (n = 162). Neither *Plasmodium* (22.1% of yearlings, 16.7% of adults; $\chi^2 = 1.1$, df = 1, $P = 0.303$, Cramér's V = 0.068), *Leucocytozoon* (10.3% of yearlings, 5.6% of adults; $\chi^2 = 1.7$, df = 1, $P = 0.191$, Cramér's V = 0.088), nor *Haemoproteus* (11% of yearlings, 8.6% of adults; $\chi^2 = 0.2$, df = 1, $P = 0.62$, Cramér's V = 0.04) infections were more likely to occur in younger birds. When we restricted comparisons between irruptive and resident chickadees to just yearlings, only *Plasmodium* infections remained more common in irruptive BCCH (40% vs. 13.2%; $\chi^2 = 11.1$, df = 1, $P < 0.001$, Cramér's V = 0.304).

DISCUSSION

This study was conducted to assess whether the presence of certain haemosporidian lineages in black-capped chickadees could be used to identify non-local birds during irruption events, and we found that parasite community variation reflects the different geographic origins of chickadees. In line with our predictions, several seemingly common lineages from local chickadees were rare or absent among irruptive BCCH, and we found at least 1 *Plasmodium* lineage that could serve as a marker for identifying these non-resident birds in SE Pennsylvania.

Lineage BT7 was the most promising marker for distinguishing irruptive birds; we never found it in resident chickadees. The use of this lineage as a marker requires the assumption that it is consistently present in the same chickadee populations beyond the 2012–2018 timescale, because lineages may be highly prevalent in some years and not others (Wilkinson et al., 2016). However, this *Plasmodium* lineage seems to be an extreme host generalist, with a Holarctic distribution and records from 64 raptor, waterfowl, and migratory passerine hosts according to the MalAvi database and abundant records in Alaskan BCCH spanning more than a decade (Loiseau et al., 2012; Oakgrove et al., 2014; Wilkinson et al., 2016). Although such a widespread lineage may be inappropriate for determining exactly where a chickadee originated, we performed a literature search of nearly 800 BT7 records in MalAvi and found that the strain primarily infects birds nesting in boreal (vs. temperate or subtropical) regions. For juvenile and nonmigratory hosts, all instances of BT7 occurred north of the north 45th parallel (45° N) (Szöllősi et al., 2011; Loiseau et al., 2012; Oakgrove et al., 2014; Podmokła et al., 2014; Wilkinson et al., 2016; Ellis et al., 2020), with exceptional records including 1 from a northern cardinal (*Cardinalis cardinalis*) in Texas (DeBrock et al., 2021) and another among juvenile *Catharus* thrushes in Vermont (Starkloff et al., 2021). Otherwise, records of BT7 in SE Pennsylvania (Galen et al., 2024) and regions such as Myanmar (Ishtiaq et al., 2007) and Colombia (Pulgarín-R et al., 2019) were exclusive to long-distance boreal-nesting migrants.

The distribution of BT7's transmission areas, its presence in yearling chickadees, and the timing of the birds' irruptive movements suggest that visiting BCCH at our sites were born in Canada, northern New York, or northern New England as opposed to other parts of Pennsylvania. Furthermore, we hypothesized that these birds obtained most (if not all) of their infections on their breeding grounds. Although we cannot completely rule out the possibility of their parasites being locally transmitted and acquired, this scenario is unlikely for 2 reasons. First, the months in which BCCH irruptions typically occur do not coincide with periods of vector activity in temperate eastern North America (Adler et al., 1982; Lysyk, 2006; Galen et al., 2024). *Culex* mosquitoes, which are among the most common *Plasmodium* vectors in temperate regions (Kim and Tsuda, 2010; Glaizot et al., 2012; Lalubin et al., 2013), enter diapause when photoperiods shorten to 12 h (Dawson et al., 2007) and daily mean temperatures drop below 10 C (Eldridge, 1987). With SE Pennsylvania annually meeting these conditions from October to March, the likelihood of *Culex* and other vectors being active or numerous enough to infect non-resident BCCH during these time frames seems low. Second, even if year-round haemosporidian transmission were possible at our sites, local insect vectors may be unsuitable for transferring lineages such as BT7 between irruptive BCCH and resident chickadees. Several authors have suggested or demonstrated the importance of vector communities in shaping local haemosporidian assemblages (Ishtiaq et al., 2008; Pagenkopp et al., 2008; Carlson et al., 2015), and some parasite lineages require particular mosquito (Culicidae), blackfly (Simuliidae), or biting midge (Ceratopogonidae) species for transmission (Santiago-Alarcon et al., 2012; Gutiérrez-López et al., 2020). Given that BT7 infects an enormous variety of birds, it seems possible that its areas of transmission are limited by vector rather than host availability, especially if some passerines carrying BT7 are migrating through Pennsylvania in August while mosquitoes are active (Galen et al., 2024). However, several widespread *Plasmodium* lineages are vector generalists compatible with multiple species or even genera of mosquitoes (Kimura et al., 2010; Njabo et al., 2011; Ferraguti et al., 2013). Thus, probably some other combination of biotic and abiotic factors prevents the BT7 lineage from being transmitted at our study sites.

Despite also being prevalent in irruptive BCCH, lineage GEOTRI09 made a less promising marker for identifying these visitors than did lineage BT7 because it occurred in a single resident chickadee and occurs extensively in other nonmigratory passerines from SE Pennsylvania (Galen et al., 2024). Why lineage GEOTRI09 infected a higher proportion of irruptive vs. resident birds could be a function of different vector communities on their respective breeding grounds, resident chickadees being more resistant to this lineage, or irruptive BCCH spending more time in microhabitats where lineage GEOTRI09 vectors occur. Although these scenarios are purely conjectural, one straightforward way to address each of them would be to collect insect vectors near birds' nest sites, screen them for haemosporidians, and do the same within BCCH populations at the northern end of this species' range (in addition to sampling the birds themselves). An ever-increasing number of researchers have isolated avian haemosporidians from wild-caught vectors (Kimura et al., 2010;

Njabo et al., 2011; Bernotienė et al., 2019; Chakarov et al., 2020; Jumpato et al., 2024), and such studies will be crucial for piecing together the interactions among host, parasite, and vector communities in systems such as ours.

Regarding other markers, we considered the possibility that some haemosporidian lineages could identify BCCH of unknown provenance as residents. However, our limited sample of irruptive birds and inability to capture them at other times of year made this question difficult to address. Lineages DUMCAR02 and COLBF21 (both *Leucocytozoon*) were absent among non-resident BCCH but also were absent or rare among residents sampled during winter months (Rice et al., 2021). Thus, it is possible that DUMCAR02 and COLBF21 lineages infect irruptive BCCH on their breeding grounds but become less detectable outside of the birds' breeding seasons because they are no longer circulating in their hosts' bloodstreams after transmission periods have ended. This trend has been commonly observed among haemosporidians and may result from the birds' immune systems actively eliminating infections over time or from parasites entering a latent or dormant phase before recrudescing in the following spring or summer (Valkiūnas, 2005; Szöllősi et al., 2016). Either scenario is plausible when considering that *Leucocytozoon* transmission periods in eastern North America typically occur in late spring and early summer when blackflies are most abundant (Bennett and Cameron, 1974; Adler et al., 1982) and that *Leucocytozoon* prevalence and parasitemia have been shown to decrease in other bird species outside of their respective breeding seasons (Deviche et al., 2001, 2010; Murata et al., 2007; Šujanová et al., 2021; Reinoso-Pérez et al., 2024).

Although our study is not the first to address parasites as potential geographic markers for birds (Fallon et al., 2006; Durrant et al., 2008; Pagenkopp et al., 2008; Santiago-Alarcon et al., 2011), no other researchers have specifically used haemosporidians to ascertain the migratory status of conspecific or congeneric birds with overlapping winter distributions. By doing so, we found that haemosporidian data can be useful for identifying some individuals with divergent migratory strategies within a host species. However, many if not most haemosporidian lineages are probably unsuitable for determining migratory connectivity between bird populations or pinpointing precise geographic origins of individual hosts. Not every bird from a certain region will test positive for a specific lineage (as shown by BT7 infecting a small subset of BCCH), and many range-restricted lineages also tend to be rare or undersampled where they occur. Some lineages may not be stable in bird populations over long periods of time or they may differ interannually in terms of prevalence (Bensch et al., 2007). However, our findings suggest that even within a single host species in a small geographic region, differences in parasite communities are associated with differences in host migratory status.

In light of our findings, we have several recommendations for further work involving haemosporidians as markers of divergent migratory behavior among birds. First, studies should address whether hosts are likely to be infected on their wintering grounds or migratory stopover sites, which will depend on phenological overlap between their annual arrival dates and periods of vector activity (which could in turn be affected by weather conditions each year). Second, researchers should consider that birds' haemosporidian communities may change over time as novel lineages spread through host populations and other lineages are eliminated. Upcoming work would greatly benefit from screening haemosporidian vectors across years, seasons, or larger geographic areas where parasite-vector dynamics may differ. This approach would not only address the most common shortcomings of avian haemosporidian studies but also bridge a major knowledge gap in our understanding of these mysterious parasites' distributions and ecological interactions.

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