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Geographic variation in malarial parasite lineages in the Common Yellowthroat (Geothlypis trichas)

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RESEARCH ARTICLE

Geographic variation in malarial parasite lineages in the common yellowthroat (*Geothlypis trichas*)

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Abstract Our current understanding of migration routes of many birds is limited and researchers have employed various methods to determine migratory patterns. Recently, parasites have been used to track migratory birds. The objective of this study was to determine whether haemosporidian parasite lineages detect significant geographic structure in common yellowthroats (*Geothlypis trichas*). We examined liver tissue or blood from 552 birds sampled from multiple locations throughout the continental United States, southern Canada, and the Bahamas. We found a 52.7% overall prevalence of haematozoan infection. We identified 86.1% of these infections to genus: 81% were *Plasmodium*; 5% were *Haemoproteus*; and 0.1% were *Leucocytozoon.* There were significant differences in the prevalence of different parasite genera among regions ($\chi^2 = 36.82$, $P < 0.0001$) and in the proportion of *Plasmodium* infections versus other parasites among regions ($\chi^2 = 35.52$, $P < 0.0001$). Sequence information identified three *Haemoproteus* lineages, two *Leucocytozoon* lineages, and thirteen *Plasmodium* lineages. Due to the low number of *Haemoproteus* and *Leucocytozoon*, only *Plasmodium* lineages were used in the geographic comparison of lineages. Six *Plasmodium* lineages were found in eight or more birds and the prevalence of these varied significantly among regions ($\chi^2 = 172.33$, $P < 0.0001$). Additionally, 45 juvenile birds were sampled to determine what parasites could be obtained in the breeding grounds and we found only one lineage. In conclusion, parasite lineages show some geographic structure, with some lineages being more geographically specific than others, but are not useful for determining migratory connectivity in this species.

Keywords *Plasmodium* · Common yellowthroat · Migratory connectivity · mtDNA · *Geothlypis*

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Introduction

Our current understanding of the migration routes of many migratory bird species is very limited. Only broad ranges for migratory birds are usually known, and rarely do we know where individuals or populations within a species go to or the path they use to travel to different areas. Webster et al.

(2002) called this 'migratory connectivity', which they defined as "the extent to which individuals from the same breeding area migrate to the same non-breeding area".

Currently, many populations of migratory birds are declining for multiple reasons, primarily habitat destruction and/or alteration (Robbins et al. 1989; Ballard et al. 2003). Knowing the migratory connectivity of species can help to identify location-specific threats to these declining migratory bird populations on the wintering and breeding grounds and provide insight regarding overall population health and survivorship (Latta and Baltz 1997). Knowing the migratory patterns of birds is also important for understanding the evolution and life history traits of migratory birds. In many studies, year round data was needed to truly understand the ecology and life history of migratory birds, which is impossible without knowing where these birds live in the various stages of their life cycle (Robinson et al. 1995; Tankersly and Orvis 2003). Knowing migratory connectivity is essential to understanding survival rates through the migrants annual cycle as well as determining the type and location of stopover sites (Sillett and Holmes 2002).

In addition, it has been shown that migrants carry pathogens during migration (Valkiunas 2004), some of which can infect humans (Gylfe et al. 2000; Peterson et al. 2003), so knowing the migratory connectivity of these birds can be important in determining the spread of a disease. Migratory birds have been shown to carry 46 known pathogens including avian malaria, Crimean-Congo hemorrhagic fever, *Escherichia coli*, *Salmonella enterica*, influenza, West Nile virus, and *Staphylococcus aureus* (for complete list see Hubalek 2004). It has been suggested that the stress of migration, which suppresses the bird's immune system, causes higher intensities of infection (Booth and Elliott 2003), which means that migrants may relapse upon completing their migration, thus allowing the pathogen to spread in the new area (Gylfe et al. 2000; Hasselquist et al. 2007).

Since migratory connectivity is such an important aspect of the ecology, behavior, and evolution of migratory bird species, many researchers have attempted to track migratory birds through a variety of ways with varying success. Initially, researchers attempted to band birds in their breeding ranges and then recapture them on the wintering ground or vice versa. However, this method has been less than ideal due to the low probability of recapturing or resighting individuals on their wintering ground or during migration (Sillett and Holmes 2002). Using satellite telemetry to track migratory patterns has worked well for large birds, such as North American ospreys (Martel et al. 2001), but it is impractical for small birds (Webster et al. 2002). Morphological characteristics have also been used to track migratory birds, but this technique only works if bird populations within a species have distinct morphological characteristics, which many do not (Webster et al. 2002; Wennerberg et al. 2002). Genetic markers have been applied to track migratory patterns. However, this method only works with rapidly-evolving loci in species with phylogeographic structure, and has had varying success depending on the bird species in question (Haig et al. 1997; Milot et al. 2000; Kimura et al. 2002; Pitra et al. 2004). It is believed that the lack of differentiation among populations is probably due to a low degree of site fidelity and high gene flow (Slatkin 2004).

A promising method of determining migratory connectivity is the use of stable isotopes. Most migratory songbirds in North America molt their feathers annually in their breeding grounds, so their new flight feathers should show the isotopic signature of their breeding ground. In most cases, researchers were able to determine migratory connectivity using stable isotopes (Hobson and Wassenaar 1997; Rubenstein et al. 2002, Marra et al. 1998; Hobson et al. 2001; Hobson et al. 2004); however, there are a number of limitations to consider such as variation in isotopic signatures with diet, altitude, location, and trophic levels (Chamberlain et al. 1997; Hobson et al. 2004; Wassenaar and Hobson 2000).

It has been suggested that parasites, specifically avian haemosporidians, can be used to track migratory birds. Multiple studies have looked at the transmission, occurrence, spatial and/or temporal variation of avian haemosporidians, but not all of these studies have linked their findings with migratory connectivity. Rintamäki et al. (1998) found differences in timing of blood parasites in willow warblers (*Phylloscopus trochilus*) during the breeding season, with *Haemoproteus* infections being more common in the beginning of the breeding season and *Leucocytozoon* infections being more common in the latter part of the breeding season. They attributed this difference in the timing of infection to the idea that these birds may have come from different subpopulations that were exposed to different parasites at different times. Hasselquist et al. (2007) found that parasite intensity of *Haemoproteus payevskyi* in the great reed warbler (*Acrocephalus arundinaceus*) decreased during the breeding season, which they interpreted to mean that the birds acquire their infection on the wintering grounds. However, while Rintamäki et al. (1998) had a large sample size, they only surveyed a single location in Finland, not various areas within the breeding range. Additionally, both of these studies used microscopy to identify infections, but more recent studies have shown that PCR techniques are much more sensitive at detecting parasite infections (Bensch et al. 2000; Richard et al. 2002; Fallon et al. 2003; Waldenström et al. 2004). Bensch and Åkesson (2003) used a PCR based method to detect both temporal and spatial change in two strains of *Haemoproteus* in Swedish willow warblers, *Phylloscopus trochilus*, over a three-year study period.

With the use of molecular techniques, Waldenström et al. (2002) found two clades of avian haemosporidians that were exclusively transmitted in Africa and at least one clade of *Haemoproteus* that appeared to be transmitted on both the wintering and breeding grounds. Fallon et al. (2006) identified twelve distinct lineages of haemosporidian parasites from 1,069 breeding black-throated blue warblers (*Dendroica caerulescens*) collected from throughout their entire eastern United States breeding range. However, since three of those lineages were common throughout the breeding grounds and accounted for 70% of all infections, parasite lineages do not appear to be useful as markers of geographic origin in this species.

The objective of this study was to determine whether haemosporidian parasite lineages (i.e. *Plasmodium*, *Haemoproteus*, and *Leucocytozoon*), as defined by mitochondrial DNA sequences, could be used to detect significant geographic structure in common yellowthroats and hence predict the locality from which a bird originated. Hellgren et al. (2007) found that *Plasmodium* was more likely to change transmission area than *Haemoproteus* and *Leucocytozoon*, which both had more restricted transmission areas. Additionally, another study found that year round transmission of malarial parasites in the blackcap, *Sylvia atricapilla* (L.), were much more geographically widespread and had higher local prevalence than those that were transmitted only during the summer (Perez-Tris and Bensch 2005). This suggests that *Plasmodium* should be more widespread, as it is more likely to be transmitted year round, and that *Haemoproteus* and *Leucocytozoon* should have a lower prevalence, but more geographically restricted. We chose the common yellowthroat (*Geothlypis trichas*) as the study subject, mainly because of its extensive breeding range, which encompasses the entire continental United States and the southern portions of Canada (http://www.mbr-pwrc.usgs.gov). Phylogeographic studies on the common yellowthroat show a deep divide in mitochondrial DNA sequences between eastern and western regions, but virtually no differentiation at a smaller scale (Ball and Avise 1992; Lovette et al. 2004). In addition, we tested juvenile birds from one of our study sites to determine if the parasites that we detected were more likely from the breeding ground or brought with the migrating birds from the wintering grounds.

Materials and methods

Sample collection

The collection localities of samples used in this study are shown in Fig. 1, and for the origin and collector of each local sample see electronic supplementary material. Note that in some cases blood-impregnated liver tissue was collected, and in other cases blood was sampled, but all samples were collected only during the breeding season (see electronic supplementary material). Since both blood and tissue were used to calculate prevalence, we compared the amplification success of the blood samples from Wisconsin with the tissue samples from Wisconsin, which was the only state that contained both tissue types. The amplification success between the two different kinds of tissue was not significantly different (Fisher's Exact $P = 0.14$; blood samples $n = 24$ out of 29; tissue samples $n = 18$ out of 18), so the two tissue types were combined for all the analyses. Ishtiaq et al. (2006) also found this for common mynas (*Acridotheres tristis*).

We obtained an additional 23 blood samples of common yellowthroats in Maine and upstate New York in early July 2005. The birds were captured in mist nets and blood was taken from the humeral vein. The blood was stored in lysis buffer and frozen until extraction.

In addition, we obtained blood samples from 47 juvenile birds (ages ranging from 6 days to approximately 27 days) from a population in Wisconsin and screened these with the same methods used for the adult birds. This was the only region where juvenile birds were available and, since the adults from this area had such a high infection rate ($\sim$89%), it seemed a good area for testing the presence of parasites in juveniles. Age was known for 38 of the 47 juveniles, and nine of these were older than 15 days post-hatching. Because all juveniles greater than 10.3 g were over 15 days of age, we were able to infer an age greater than 15 days for four of the juveniles of unknown age. This suggests that nearly a third ($n = 13$) of the juveniles sampled had more than 15 days of exposure to infection. While a *Plasmodium* or *Haemoproteus* infection takes from 9 to 14 days to become detectable in the blood (Valkiunas 2004; Fleischer, unpublished), *Leucocytozoon* infections can be detected sooner (Merino and Potti 1995).

The samples were divided into five geographic regions: northwest, southwest, north central, southeast, and northeast. The regions were determined based on the major terrestrial ecosystem associated with each region as well as the distribution of the samples. The northwest region, mainly evergreen forest, included all samples from Canada-Alberta, Canada-British Columbia, Idaho, Montana, Oregon, and Washington. The southwest region, an arid area with few large trees and more desert-type habitat, included all samples from Arizona, California, Kansas, New Mexico, Nevada, and Texas. The northeast region, mainly deciduous forest, included all samples from Canada-Newfoundland, Delaware, Maryland, Maine, and New York. The southeast region, a warmer and wetter forested area, included all samples from Alabama, Arkansas, Florida, Louisiana, Mississippi, North Carolina, South

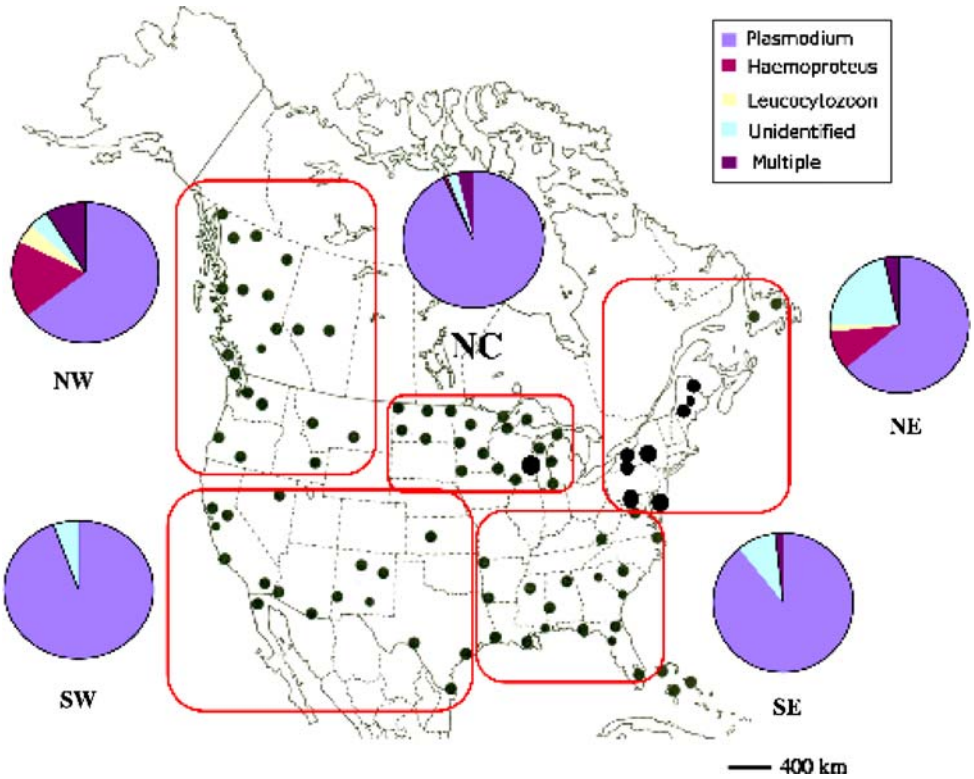

Fig. 1 Map of collection sites. The pie graphs are arranged with their corresponding region (NW = northwest; SW = southwest; NC = north central; SE = southeast; NE = northeast). Within the pie graphs, purple is *Plasmodium*, pink is *Haemoproteus*, and yellow is *Leucocytozoon*; identifications based on a combination of restriction digest data and sequence data. The size of the circle corresponds to the number of samples taken (small circle = 1–2 samples, medium circle = 5–7 samples, large circle = >10 samples)

Carolina, and Virginia. The north central region, a mix of forested lakeside habitat and prairie, included all samples from Michigan, Minnesota, North Dakota, and Wisconsin.

DNA analysis

DNA was extracted from liver and blood samples using Qiaquick Dneasy kits (Qiagen, Valencia, California). The samples were screened for infection based on the presence or absence of a PCR amplified product of a conserved region of non-coding mtDNA sequence from the parasite using the primers 213F/372R as described in Beadell and Fleischer (2005) and of a less conserved region of cytochrome b using the primers F2/R2 as described in Beadell et al. (2004). A positive infection was indicated by the presence of an appropriately sized band from either assay on a gel after electrophoresis. After the initial screening, all birds that showed a positive result from either primer set were used in the analysis. Negative controls were included in all extraction and PCR bouts to check for contamination. A positive control, a blood sample containing *Plasmodium relictum*, was also included in one PCR reaction per bout.

To determine the genus of the parasite, the 213F/372R PCR product from infected birds was surveyed via a restriction enzyme analysis as described in Beadell and Fleischer (2005). This restriction enzyme analysis assigns parasite lineages to *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* and will indicate multiple infections between genera.

To ensure that all DNA extractions were successful and thus not the reason for the inability to amplify parasite DNA, 268 base pairs of avian mtDNA was also amplified using the primers cytb-2RC and cytb-wow (Dumbacher et al. 2003). Any sample that did not amplify with this primer set was excluded completely. This was only the case with three of the juvenile samples.

The prevalence of haemosporidian infection was calculated for all birds as well as for each of the five regions. All infections detected with either 213F/372R or F2/R2 were used to calculate total prevalence. We combined the restriction enzyme digest data with the sequence data to determine the total number of *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* per region. We used Statview 4.5 to calculate a χ^2 value comparing the overall parasite prevalence among regions, the prevalence of *Plasmodium* among regions, and the frequency of each genus (when known) among regions to test if the prevalence of haplotypes was equal.

For sequencing, a larger fragment of cytochrome b (533 bp) was amplified with the primers 3760F/4292rw2 and the PCR protocol is described in Beadell et al. (2004). The PCR product was sequenced directly in both the forward and reverse directions with the same primers. If this fragment of cytochrome b did not amplify, we attempted to amplify smaller fragments within this region

using a variety of other primer sets (F3/4292rw2, Beadell et al. 2004; FIFI/4292rw2, Ishtiaq et al. 2006) and these were also directly sequenced. The smallest piece of cytochrome b that was used in the phylogenetic analyses was 295 bp. Some parasites remained unknown due to lack of large piece amplification or lack of amplification with 213F/372R. For those that we only had amplification with F2/R2, this small fragment (91 bp) was sequenced to determine the parasite to genus; however, this small amount of information was only included in prevalence data and not in the phylogenetic analysis. All fragments were sequenced with Big Dye Terminator Cycle Sequencing Kit on an ABI 3100 Sequencer (Applied Biosystems, Inc).

Phylogenetic and statistical analysis

We were able to successfully sequence 73.9% of the parasites from the positive birds ($n = 215$), a level comparable to other studies of avian blood parasites (Beadell et al. 2004; Fallon et al. 2006; Ishtiaq et al. 2006). Parasite lineages were determined by aligning and comparing sequences with SEQUENCHER (version 4.1) and McClade (4.0). Parasite lineages were defined as sequences that differed by two or more base pairs. Relationships among parasite lineages were assessed using a minimum evolution criterion with a Kimura 2-parameter evolutionary model in the program PAUP* 4.0 (Swofford 1999). Support for the internal nodes of the tree was determined by 1,000 bootstrap repeats using a full heuristic search after starting with a neighbor-joining tree. We determined the genus of each clade by including known *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* sequences from GenBank. The frequencies of lineages among regions were compared to determine if there was any geographic structure in the parasite lineages. There were six lineages found in eight or more birds and we performed contingency-table χ^2 tests to determine if the lineages varied in frequency geographically.

Results

Prevalence

We screened a total of 552 birds and found 52.7% overall parasite prevalence ($n = 291$). Prevalence differed significantly among the five regions (Table 1; $\chi^2 = 86.4$, $P < 0.0001$), even after removing the southwest region, which had the lowest prevalence ($\chi^2 = 38.2$, $P < 0.0001$).

Of the 291 birds positive for infection, 81% of the infections were *Plasmodium* ($n = 237$), 5% of the infections were *Haemoproteus* ($n = 15$), and 0.1% of the infections were *Leucocytozoon* ($n = 3$). The remainder of the infections ($n = 23$) could not be determined. There were 13 multiple infections found. Most multiple infections were determined either with double sequence in chromatograms or double bands after restriction enzyme digest. Of those multiple infections, five were double *Plasmodium* lineages, one was a double *Haemoproteus* lineage, and five were mixed *Plasmodium* and *Haemoproteus*. Two of the *Plasmodium* and *Haemoproteus* mixed infections were found when the restriction enzyme digest showed *Haemoproteus* but *Plasmodium* was sequenced. Another mixed infection was found when a sequencing primer set amplified *Haemoproteus*, but the screening primer amplified *Plasmodium*. The sequences obtained from these three mixed infections were included in the phylogenetic analysis, but all other multiple infections were removed from the phylogenetic analysis. The prevalence of each genus by region is shown in Fig. 1. The proportion of *Plasmodium* infections versus all other infections (*Haemoproteus*, *Leucocytozoon*, multiple, and unidentified combined) was significantly different among regions ($\chi^2 = 36.52$, $P < 0.0001$). There were not enough samples of the other two genera to singly compare their prevalence among regions. When we compared the prevalence of only the infections that we were able to identify to genus (excluding all unknown infections and multiple infections), we also found that there was a significant difference in the prevalence of the different genera among the regions ($\chi^2 = 36.82$, $P < 0.0001$).

Of the 44 juveniles screened for parasite infection, 28 were 1–14 days post-hatch, seven were 15–20 days post-hatch, and five were 21–27 days post-hatch. The remainder ($n = 4$) were of unknown age. Only two juveniles were positive for parasite DNA. Both juveniles had the same *Plasmodium* parasite strain, P4, which was only found in four adult birds in the same region, the North Central region. In addition, both juveniles were approximately 27 days old, so they were well past the fledgling stage (approximately 10 days for common yellowthroats).

Phylogenetic analysis

Based on our sequences we obtained eighteen lineages of haematozoan parasites (Fig. 2; GenBank Accession EU328160-EU328181). Lineages P1, P2, and P3 made up one well-supported clade. Lineages P9A, P9B, P10, P11, and P12 made up another well-supported clade. There was not strong bootstrap support for basal nodes among all *Plasmodium* lineages. We obtained only three lineages of *Haemoproteus* from our sequences; one was only found in the Bahamas while the others were only found in the

Table 1 The total number of birds from each region and corresponding prevalence for total *Plasmodium*, *Haemoproteus, Leucocytozoon*, multiple infections, and unidentified haemosporidian infections

Region	Total	Positive	Plasmodium	Haemoproteus	Leucocytozoon	Multiple	Unidentified
NW	90	45	0.64	0.18	0.04	0.09	0.04
SW	91	18	0.94	0.00	0.00	0.00	0.06
NC	144	113	0.93	0.01	0.00	0.04	0.03
NE	127	56	0.63	0.09	0.02	0.05	0.21
SE	86	55	0.89	0.00	0.02	0.02	0.09
Total	538	287					

Samples from the Bahamas were excluded

northeast. Only two lineages of *Leucocytozoon* were found; one lineage was found solely in the northeast while the other was only found in the northwest. While we do not know whether each of our lineages corresponds to a species, previous data comparing mtDNA and nuclear markers suggest that this may be the case (Bensch et al. 2004).

Upon reviewing the sequences it appeared as though our sequencing primers may have been biased towards amplifying *Plasmodium* sequences. We obtained sequences from 90% of the infections identified as *Plasmodium*, but only 25% of the *Haemoproteus* infections. We did sequence 100% of the *Leucocytozoon* infections, but we only recovered three of these. We infer that this discrepancy was caused by a bias in our sequencing primers. However, because of the small number of *Haemoproteus* and *Leucocytozoon* infections found in common yellowthroats, both *Haemoproteus* and *Leucocytozoon* were excluded from the geographic lineage comparisons.

We found thirteen lineages of *Plasmodium* in common yellowthroats in North America (Fig. 3). The samples from the Bahamas were excluded from regional comparisons since they were the only samples that were not from the North American continent. The prevalence of each *Plasmodium* lineage varied greatly. Six lineages were found in eight or more birds. When we compared the geographic distribution of those six lineages, we found that they were significantly different among regions ($\chi^2 = 172.33$, $P < 0.0001$). Lineage P6 was the most prevalent lineage found in 100 birds, mostly in the north central region, though it was also found less commonly in the northwest, southwest, and southeast. Lineage P4 was the second most prevalent lineage found mostly in the southeast, but also found in the northeast and north central. Lineage P1 was relatively evenly spread across all five regions. Lineage P9 was found mostly in the northeast, but it was also found in the north central, southwest, and southeast. Lineage P2 was mostly in the southwest and north central, but also seen in the northwest. Lineage P5 was found mostly in the northwest, but there was one occurrence in the northeast. The remaining lineages were found only a few times, and were not in sufficient frequency for comparison.

Since parasites have shown temporal variation in other species (Bensch and Åkesson 2003), we compared the lineages found in Wisconsin in 1991–95 with those found in 2005 to determine if there had been a change in lineage frequency over time. From 1991 to 95, 84% of the lineages found were P6. In 2005, 88% of the lineages found were P6. Thus, there was no significant change in the frequency of typed lineages over that 14-year period ($\chi^2 = 1.32$; $P = 0.72$). Unfortunately, we did not have information from multiple years for any other area, so we were unable to do this comparison for other regions.

We compared our sequences to those in Genbank and found that ten of the lineages we found had been obtained previously in other studies (Table 2). These lineages were all found in North America, except one lineage that was also found in South Korea and where geographic location was not provided. The lineages were also mostly found in other warblers (Parulidae), but they were also found in multiple non-migratory species of both North and South America.

Discussion

This study examines whether haemosporidian parasites can serve as markers of geographic origin that can be used for tracking the migratory patterns of the common yellowthroat. Our results suggest that there is some geographic structuring of *Plasmodium* lineages, but their utility for determining migratory connectivity is limited.

A potentially confounding issue we discovered was that our sequencing primers preferentially amplified *Plasmodium* over other parasite lineages and, as we found very low numbers of *Haemoproteus* and *Leucocytozoon* infections with our unbiased screening primers, our analyses of geographic structure were limited to *Plasmodium*. Although our sequencing primers may have been biased towards *Plasmodium*, we do not believe that our screening primers were biased. First, we used two different primers that amplify two different regions of mtDNA, a conserved non-coding region and a more variable cytochrome b

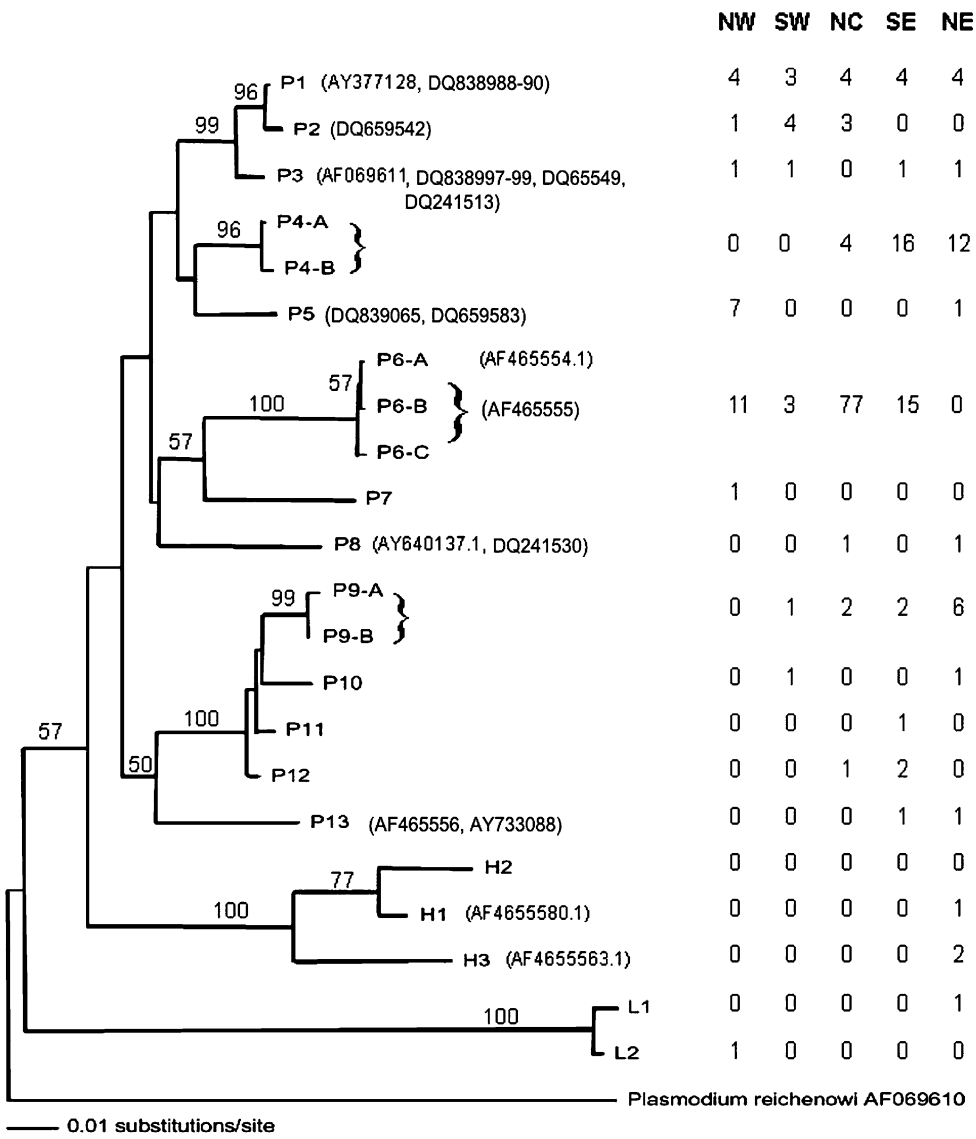

Fig. 2 A minimum-evolution tree showing the relationships between all *Plasmodium*, *Haemoproteus*, and *Leucocytozoon* lineages found. Bootstrap values under 50 are not shown. The numbers on the right correspond to the number of times that lineage was found in that region. Haplotype H2 was found only in the Bahamas samples, which were not used in the regional comparisons. The lineages we found that have matches in Genbank have the corresponding Genbank accession number shown

region, (the first of these was designed to amplify all three genera, Beadell and Fleischer 2005), to ensure detection of as many infections as possible. These primers also amplify relatively small fragments (91bp for F2/R2 and 160bp for 213F/372R).

Parasite prevalence among regions

We were able to assess the prevalence of all three genera (*Plasmodium*, *Haemoproteus*, and *Leucocytozoon*) in each region, and made an interesting discovery that there are highly significant differences in prevalence among regions (Table 1). There are a number of possible explanations for the difference in frequency across regions. The overall prevalence of the three different parasite genera may be different among the five regions due to differences in the abundance of vectors among regions (Super and van Riper III 1995; Klei and DeGuisti 1975; Atkinson et al. 1988). Sol et al. (2000) showed that prevalence of parasites can vary greatly between populations that are geographically close due to the density of the vector and when vector densities are high, transmission is also high. The southwest region had the lowest prevalence (19.7%), possibly because of a lower density of vectors. Most of this region is very dry, which suggests that it is not a very good breeding ground for vectors of haematozoa, which all require water during their larval stages (Atkinson and van Riper III 1991; Greiner et al. 1975). It is also possible that the southwest common yellowthroats have evolved a different immune response that causes them to be less vulnerable to haematozoan infection. This phenomenon has been shown among different populations of Hawai'i 'amakihi (*Hemignathus virens*), where populations in lower altitudes

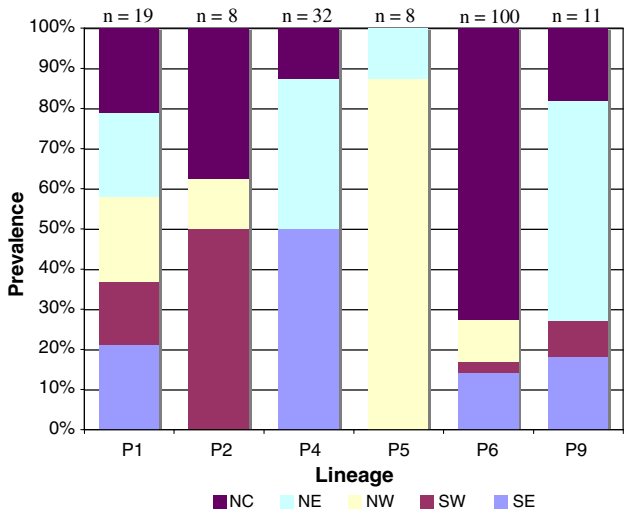

Fig. 3 Prevalence of the six *Plasmodium* lineages with eight or more individuals found in each of the five regions of North America

appear to have a different immunological response to *Plasmodium relictum* than those that live at higher altitudes (Jarvi et al. 2001; Woodworth et al. 2005).

It is possible that our results could have been affected by changes in prevalence of these parasites over time. Our samples were collected from various years and all years were combined for this study. This is because using parasite lineages to track migratory birds would only be useful if the parasite lineages showed temporal stability since it would be impossible to resample a birds' entire breeding ground every year. For the one area where we had samples from different years for comparison, we did not find any difference in parasite lineages in two samples collected 14 years apart. Another study conducted by Fallon et al. (unpublished) for another North American bird, the black-throated blue warbler (*Dendroica caerulescens*), showed no temporal variation with year-by-year sampling in the same location. Additionally, Bensch et al. 2007 found no change in parasite lineage frequency over a 17 year period in a population of great reed warblers, *Acrocephalus arundinaceus*. However, Bensch and Åkesson (2003) found some spatial and temporal differences in the distribution of *Plasmodium* and *Haemoproteus* in a European migratory bird, the willow warbler. It is possible that many factors including species, environment, vector, etc. affect whether or not a parasite lineage is temporally stable and more research is needed in this area to understand why lineage frequencies appear stable in some populations and not others.

In previous studies, results obtained from testing juveniles less than one month old for avian haematazoa have varied. Cosgrove et al. 2006 found only a single *Leucocytozoon* infection in 195 samples from 14-day-old blue tits (*Cyanistes caeruleus*) and no *Plasmodium* or *Haemoproteus* infections. Similarly, no evidence of *Haemoproteus* was found in 25–28 day post-hatch Purple Martins (*Progne subis*; Davidar and Morton 1993). Merino and Potti (1995) tested 96 samples of 13-day-old Pied Flycatchers (*Ficedula hypoleuca*). They found no *Haemoproteus* infections but one *Leucocytozoon* infection and twenty *Trypanosoma* infections. Hasselquist et al. (2007) found no *Haemoproteus payevsky* infections in almost 100 juvenile great reed warblers between 4 and 9 weeks old. Contrary to this, Weatherhead and Bennet (1992) reported high levels of avian haematozoa in hatch-year brown-headed cowbirds after fledging (10–11 days for brown-headed cowbirds). Since we detected a very low number of juvenile infections, there is little we can say about which parasites are transmitted on the breeding ground. This requires a great deal more research since we were only able to screen a small number of juveniles. All we can say at this time is that P4 is being transmitted on the breeding ground, but this does not mean that it is not also transmitted in the wintering ground. Additionally, through matching our lineages to those previously submitted to Genbank, we found two lineages, P3 and P8, which were found in resident birds of South America, demonstrating that these two lineages must be transmitted in the wintering range, but could also be transmitted in the breeding range.

Lineage frequency among regions

Using parasite lineages to track migrations or histories of populations is a relatively new approach and has had varying success. Falush et al. (2003) used *Helicobacter pylori*, a gastric pathogen, to explain the migratory movements of humans that could not be explained with human DNA. Biek et al. (2006) used the quickly evolving feline immunodeficiency virus to look at a recently expanded population structure of cougars (*Puma concolor*) in Montana. By studying the virus they were able to find geographic structure in the cougar populations that was not found from DNA analysis. However, Fallon et al. (2006) was unable to find geographic structure in haemosporidian lineages in black-throated blue warblers. While we were not able to find definite geographic structure, our results showed more geographic structure than Fallon et al. (2006), which is probably due to the fact that common yellowthroats have a more extensive breeding range than black-throated blue warblers. Another study on Scandanavian willow warblers also found no geographic structuring of *Plasmodium* or *Haemoproteus* (Bensch and Åkesson 2003).

The fact that some *Plasmodium* lineages are more abundant than others may be due to the differential ability of the vectors to transmit some lineages better than others. Some vectors have the ability to transmit more than one parasite, but they transmit some parasites more effectively than others (Alavi et al. 2003). There also may be

Table 2 The number of base pair matches, parasite species, host species, host family, location at which the host species was collected, and the publication associated with the lineages we found that matched those from Genbank

Lineage	Genbank number	# bp match	Parasite species	Host species	Host family	Location found	Publication
P1	AY377128	533	*Plasmodium cathemerium*	Unknown		Unknown	Wiersch et al. 2005
	DQ838990, DQ838989, DQ838988,	533	*Plasmodium* sp. P1	***Cardinalis cardinalis***, *Thryomanes bewicki*, ***Passer domesticus***, *Carpodacus*	Fringillidae, Troglodytidae, Passeridae	USA	Beadell et al. 2006
P2	DQ659542	533	*Plasmodium* sp. P4	*Geothlypis trichas*	Parulidae	USA	Beadell et al. 2006
P3	AF069611	533	*Plasmodium elongatum*	***Passer domesticus***	Passeridae	North America	Escalante et al. 1998
	DQ838999, DQ83998, DQ838997	533	*Plasmodium* sp. P11	***Tangara preciosa, Pseudoleistes guirahuro***, *Troglodytes aedon*	Thraupidae, Icteridae, Troglodytidae	Uruguay	Beadell et al. 2006
	DQ659549	533	*Plasmodium* sp. P11	*Geothlypis trichas*	Parulidae	USA	Beadell et al. 2006
	DQ241513	533	*Plasmodium* sp. U6	***Gnorimopsar chopi, Pseudoleistes guiahuro, Tangara preciosa, Stephanophorus diadematus***, *Troglodytesaedon*, ***Turdus rufiventris***	Icteridae, Thraupidea, Troglodytidae, Turdidae	Uruguay	Durrant et al. 2006
P5	DQ839065	533	*Plasmodium* sp. P34	*Carpodacus erythrinus*	Fringillidae	South Korea	Beadell et al. 2006
	DQ659583	533	*Plasmodium* sp. P34	*Pluvialis fulva*	Charadriidae	USA	Beadell et al. 2006
P6A	AF465554	533	*Plasmodium* sp. haplotype 54	*Helmitheros vermivorus, Dendroica magnolia*	Parulidae	Missouri	Ricklefs and Fallon 2002
P6B	AF465555	533	*Plasmodium* sp. haplotype 55	***Baeolophus bicolor***	Paridae	Missouri	Ricklefs and Fallon 2002
P8	AY640137	331	*Plasmodium* sp. H1	*Dendroica petechia*	Parulidae	Ithaca, NY	Szymanski and Lovette 2005
	DQ241530	533	*Plasmodium* sp. B23	***Volatinia jacarina, Cacicus cela, Cacicus haemorrhou, Diopsittaca nobilis***	Emberizidae, Icteridaea, Psittacidae	Guyana	Durrant et al. 2006
	DQ241530	533	*Plasmodium* sp. B23	*Zonotrichia capensis*, ***Cranioleuca pyrrhophia, Gnorimopsar chopi***, *Basileuterus culcivorous, Basileuterus leucoblepharus*	Emberizidae, Furnariidae, Icteridae, Parulidae	Uruguay	Durrant et al. 2006

Table 2 continued

Lineage	Genbank number	# bp match	Parasite species	Host species	Host family	Location found	Publication
P13	AF465556	533	*Plasmodium* sp. haplotype 56	*Passerina cyanea*	Fringillidae	Missouri	Ricklefs and Fallon 2002
	AY733088	533	*Plasmodium relictum*	*Spheniscus demersus*	Spheniscidae	Baltimore zoo	Beadell and Fleischer 2005
H1	AF465580.1	362	*Haemoproteus* sp. haplotye 32	*Dendroica pensylvanica*	Parulidae	Missouri	Ricklefs and Fallon 2002
H3	Af465563.1	533	*Haemoproteus paruli* haplotype	*Dendroica dominica*, *Parula americana*	Parulidae	Missouri	Ricklefs and Fallon 2002

Only 100% matches are included. All nonmigratory species are bolded

competition during transmission between species of *Plasmodium* within the same mosquito (Paul et al. 2002). However, this may be difficult to demonstrate without a better knowledge of vector abundance and distribution.

It seems likely to us that the differences in *Plasmodium* lineages throughout North America may be due to the combination of vector distribution and host movement. This may also explain why most of the lineages were concentrated in one region, but also found uncommonly elsewhere. Pérez-Tris and Bensch (2005) found a positive relationship between dispersal distance of the blackcap, *Sylvia atricapilla*, and the local transmission of *Haemoproteus* and *Plasmodium*. Other studies have also shown that host movement increases parasite range with the parasites of sheep (Blouin et al. 1995), cattle (Blouin et al. 1995), and salmon (Criscione and Blouin 2004). Waldenström et al. (2002) also found multiple avian haemosporidian lineages in both the wintering and breeding ground of European migrants, showing that the combination of bird migration and competent vectors can spread these parasites.

In conclusion, while avian parasite lineages show some geographic structure in common yellowthroats, it is not to the extent necessary for use as a marker of migratory connectivity. The geographic variation we found did allow some discrimination of origin, but is limited to a few particular *Plasmodium* lineages. As multiple studies have shown that *Plasmodium* and *Haemoproteus* are not localized or species-specific (Waldenström et al. 2002; Bensch et al. 2000), we suggest further research focus on parasites that are more species-specific, more geographically localized, vertically-transmitted, and/or more rapidly evolving in order to find sufficient geographic structure for determining a migratory bird's origin (Wirth et al. 2005).

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