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Parasite assemblages distinguish populations of a migratory passerine on its breeding grounds

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Abstract

We attempted to establish migratory connectivity patterns for American redstarts *Setophaga ruticilla* between their breeding and wintering grounds by characterizing the composition of their haematozoan parasite assemblages in different parts of their range. We detected significant but limited geographic structuring of haematozoan parasite lineages across the breeding range of the redstart. We found that redstarts from the south-eastern (SE) region of the breeding range had a significantly different haematozoan parasite assemblage compared with populations sampled throughout the rest of the breeding range. Evidence using stable isotopes from feathers previously demonstrated that redstarts from the SE of the breeding range also have a unique and separate wintering range. Thus, although two methods of estimating migratory connectivity have now both shown the SE US breeding sub-population of redstarts to be distinct from other populations on both the breeding and wintering grounds, conclusive migratory connectivity for this species as a whole could not be established.

Introduction

Despite intense interest, we still lack understanding of how populations of many migratory species are geographically structured throughout the year (Webster *et al*., 2002; Norris *et al*., 2006b). Conditions on the wintering grounds are known to influence individuals and populations in subsequent breeding seasons, and birds may face differing selective pressures in their breeding and non-breeding ranges (Marra, Hobson & Holmes, 1998; Studds & Marra, 2005; Webster & Marra, 2005). Therefore, for a complete understanding of migratory animal ecology, evolution and conservation, it is essential that all phases of the migrant's life, including breeding, migratory and wintering periods, are linked.

Determining wintering locations and migratory connectivity has proven to be a challenge when dealing with small-bodied passerines. Traditional techniques such as mark–recapture have a low success rate, and some newer technologies like satellite transmitters are not suited to small-bodied animals (reviewed in Webster *et al*., 2002; Hobson, 2003). One useful technique for determining migratory connectivity is examining patterns of stable isotopic signatures in tissues or feathers (Hobson & Wassenaar, 1997; Marra *et al*., 1998; Hobson, 2005; Norris *et al*., 2006*a*). However, both Hobson (2005) and Norris, Wunder & Boulet (2006*b*) noted several potential sources of error with isotopic data, for example, variability in stable hydrogen isotope compositions, and recommended confirmation of results by incorporating data from other tracking methods such as satellite telemetry, or genetic data (e.g. Dockx *et al*., 2004; Dunn *et al*., 2006).

Endo-parasites offer another potential tool for measuring migratory connectivity, either through phylogeographic variation of individual parasite species or through geographic variation in the assemblage of parasite species in a particular host. Because the generation times of many parasites are short relative to those of their hosts, variation in genetic markers of parasites could become localized within host populations (Ricklefs & Fallon, 2002). Parasite lineages might also become localized with respect to the distribution of their vectors, and so have the potential to reveal fine-scale geographical information about hosts (Webster *et al*., 2002; but see Ricklefs *et al*., 2005*a*). When parasites are localized within the breeding range and are also present in the wintering population, or vice versa, it is possible to connect breeding and wintering populations and therefore establish migratory connectivity using parasite

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data alone. This information can also be combined with other methods of tracking the migratory movements of small passerines to improve our understanding of migratory connectivity for these species throughout the year.

DNA sequencing has shown that avian haematozoan parasites are highly diversified and exhibit varying levels of host specialization. Although these parasites are distributed throughout the world, except for Antarctica (Valkiunas, 2005), some genetically distinguishable lineages tend to be localized geographically (Ricklefs *et al.*, 2005*a*; Beadell *et al.*, 2006; Kimura, Dhondt & Lovette, 2006). Geographically localized populations of hosts could carry disparate parasite assemblages. If these parasites could be recognized in individuals throughout the year, one could connect populations on the tropical wintering grounds and temperate breeding grounds (Ricklefs *et al.*, 2005*a*).

Localizing host populations through their parasites has been attempted in few avian species. Haematozoan parasites of two Nearctic–Neotropical migratory warblers, the Black-throated blue warbler *Dendroica caerulescens* (Fallon, Fleischer & Graves, 2006) and the Common yellowthroat *Geothlypis trichas* (Pagenkopp *et al.*, in press) do not exhibit geographical structure. *Plasmodium* lineages have been found to differ between western and eastern US populations of House finches *Carpodacus mexicanus* (Kimura *et al.*, 2006); however, these birds are short-distance migrants.

In this study, we investigated the geographical structuring of haematozoan parasites of American redstarts *Setophaga ruticilla* (hereafter 'redstart') across their breeding range and in several wintering locations. Redstarts are long-distance migrants between their breeding range in eastern North America and their wintering range in Central America and the West Indies. Although banding data have not revealed connections between breeding and wintering populations, geographical structuring has been suggested by stable isotope analysis (Norris *et al.*, 2006*a*). Given the availability of independent data on migratory connectivity, the redstart is an excellent species in which to test the feasibility of using geographic structure in parasite populations or assemblages to assess the relationship between breeding and wintering areas. Accordingly, we have surveyed the haematozoan parasite assemblages of redstarts sampled across the breeding range and wintering grounds, to determine whether parasites exhibit geographical structuring concordant with that revealed by stable isotope analysis.

Methods

Study species

The redstart is a small (9 g) Nearctic–Neotropical migratory passerine that breeds in North America from British Columbia in the north-west to Louisiana in the south-east (Sherry & Holmes, 1997; Sauer, Hines & Fallon, 2004). Redstarts breed in mixed deciduous–coniferous forests and then migrate south to spend the winter in dry forests, lowland forests or mangroves in southern Florida, Central America and northern South America, Mexico, the Bahamas and the Caribbean (Sherry & Holmes, 1997; Norris *et al.*, 2006*a*).

Parasite sampling and screening

Redstarts were captured using mistnets and playback and sampled for blood. Sampling locations and sample sizes are summarized in Fig. 1. DNA samples were also obtained from other researchers to supplement our geographic sampling (combined total $n = 437$ birds). Of 437 redstart samples collected between 2001 and 2006, 68 were obtained on the wintering grounds and the remainder from breeding populations in North America (for locations see Fig. 1a). We extracted host and parasite DNA from the lysis-buffer-preserved samples using the manufacturer's protocols supplied with Qiagen DNeasy kits. We verified successful DNA extractions for samples by amplifying avian cytochrome *b* (cyt *b*) DNA using primers cyt *b*-2RC and cyt *b*-wow (268 bp, Dumbacher, Pratt & Fleischer, 2003).

We screened each sample four times using different primer sets designed to amplify the blood parasites *Plasmodium* and *Haemoproteus*. The use of different primer sets allows for detection of multiple genera of haematozoa given sequence variation at the priming sites, and also maximizes the chances of amplifying all infections. Although our primer sets were sensitive to small quantities of parasite DNA (Beadell & Fleischer, 2005), success can be reduced by large quantities of host DNA or DNA degradation. Screening samples more than once considerably reduced the occurrence of false negatives and ensured that a realistic picture of infection prevalence was obtained (Beadell *et al.*, 2004; Beadell & Fleischer, 2005). Short fragments were initially amplified using each of four primer sets: F2/R2 (132 bp, mtDNA cyt *b*); 850F/1024R (167 bp, mtDNA COIII, Beadell *et al.*, 2004); 213F/372R (160 bp mtDNA cyt *b*, Beadell & Fleischer, 2005) and 343F/496F (154 bp mtDNA rRNA, Fallon *et al.*, 2003). PCR typically followed conditions developed for 'ancient' DNA, to increase the probability of successful amplification of degraded samples or samples with few parasitemia (Fleischer *et al.*, 2000). Multiple positive and negative controls were included in every PCR. Samples that produced UV-visible bands after electrophoresis of the PCR product, indicating infection with one or more haematozoan genera, were then re-amplified with primers designed to target longer fragments of mtDNA cyt *b*: 543F/926R (382 bp) and 413F/926R (592 bp) (see Ricklefs *et al.*, 2005*b*). PCR products that produced the largest fragment for a sample were purified using Qiaquick kits (Qiagen Inc., Valencia, CA, USA) and bidirectionally sequenced on an ABI 3100 Sequencer (Applied Biosystems, Foster City, CA, USA). High quality sequences were assembled, aligned and edited using Sequencher v 4.1.

Where possible, we identified the genus of haematozoan present (*Plasmodium* or *Haemoproteus*) using both the phylogenetic alignment of sequences and the results of a restriction enzyme test on positive amplifications of 213F/372R (Beadell & Fleischer, 2005). However, because of problems associated with degraded or low-copy parasite

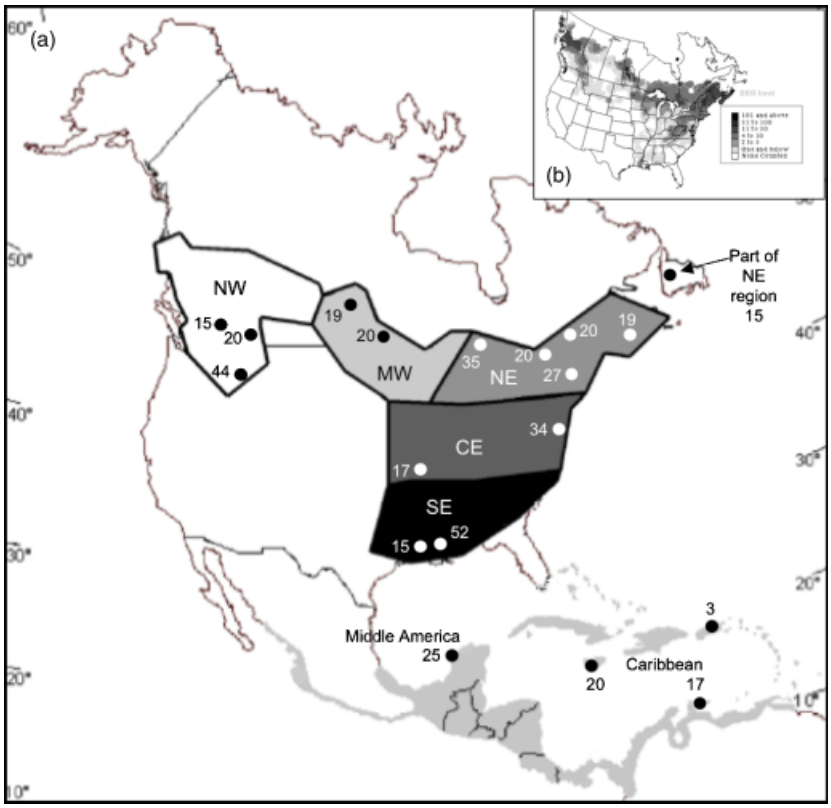

Figure 1 (a) Map showing approximate redstart *Setophaga ruticilla* breeding ground distribution in North America, sample sizes and *a priori* geographic sampling regions. Redstart wintering ground distribution are shaded grey areas across Central and South America. Adapted from Norris *et al.* (2006*a*). (b) American redstart breeding ground distribution and density from Gough *et al.* (1998).

DNA inherent in haematozoan studies (Jarvi, Schultz & Atkinson, 2002; Beadell *et al.*, 2004; Bensch *et al.*, 2004; Ricklefs *et al.*, 2005*b*), not all infected samples could be identified to genus.

Phylogenetic analyses

Samples with sequences exceeding 337 bp were used to estimate parasite phylogenetic relationships. The average sequence length included in the final tree was 503 bp. We used MacClade v. 4.06 (Maddison & Maddison, 2000) to detect unique haplotypes; one or more base differences between sequences defined a unique haplotype, and we combined haplotypes from all regions into a single dataset. For phylogenetic reconstruction, we used Modeltest v 3.6 (Posada & Crandall, 1998) to select the best model of nucleotide substitution. Both the hierarchical likelihood ratio tests (hLRTs) and the Akaike information criterion (AIC) returned the same model, GTR + G, which we then implemented in PAUP* (Swofford, 1999). We reconstructed a phylogenetic tree using maximum likelihood, and calculated bootstrap support for nodes using a heuristic search with 100 replicates. To identify genera, known examples of *Haemoproteus* (GenBank accession numbers: AY817749 and DQ321728) and *Plasmodium* (GenBank accession numbers: AY733090 and AF069611) species were included. When we recovered a sequence that matched a recorded GenBank sequence, we indicated the sequence name in the tree and determined whether the range of the recorded host species overlapped that of the redstart. We rooted the tree with a mammalian *Plasmodium falciparum* cyt *b* sequence (GenBank accession number: DQ642845), following the Perkins & Schall (2002) phylogeny.

Statistical analyses

Samples were assigned *a priori* into one of five geographic regions on the breeding grounds and two geographic regions on the wintering grounds, based on the rationale of Norris *et al.* (2006*a*), which used redstart breeding density (Gough, Sauer & Iliff, 1998; see Fig. 1b) and differing levels of stable isotopic hydrogen compounds across the continent to delineate potentially distinct populations. Basic statistics were performed using SAS v 9.1. χ^2 or Fisher's exact tests were used to test heterogeneity in prevalence and in the genera of parasites detected between regions. We used EstimateS v 7.5 (Colwell, 2005) to calculate a Chao2 estimate of parasite lineage richness, which is appropriate for presence/absence data and small sample sizes. We used this statistic to determine the total number of haematozoan lineages that could be expected from our available sequence data. Additionally, we calculated Chao2 estimates where appropriate for the total number of lineages of haematozoa recovered from each geographic region on the breeding grounds, to determine our success in sampling all available haematozoan taxa.

Finally, we used a probability approach to estimate whether region-specific clades of parasites were likely to occur by chance alone. We randomly assigned geographic

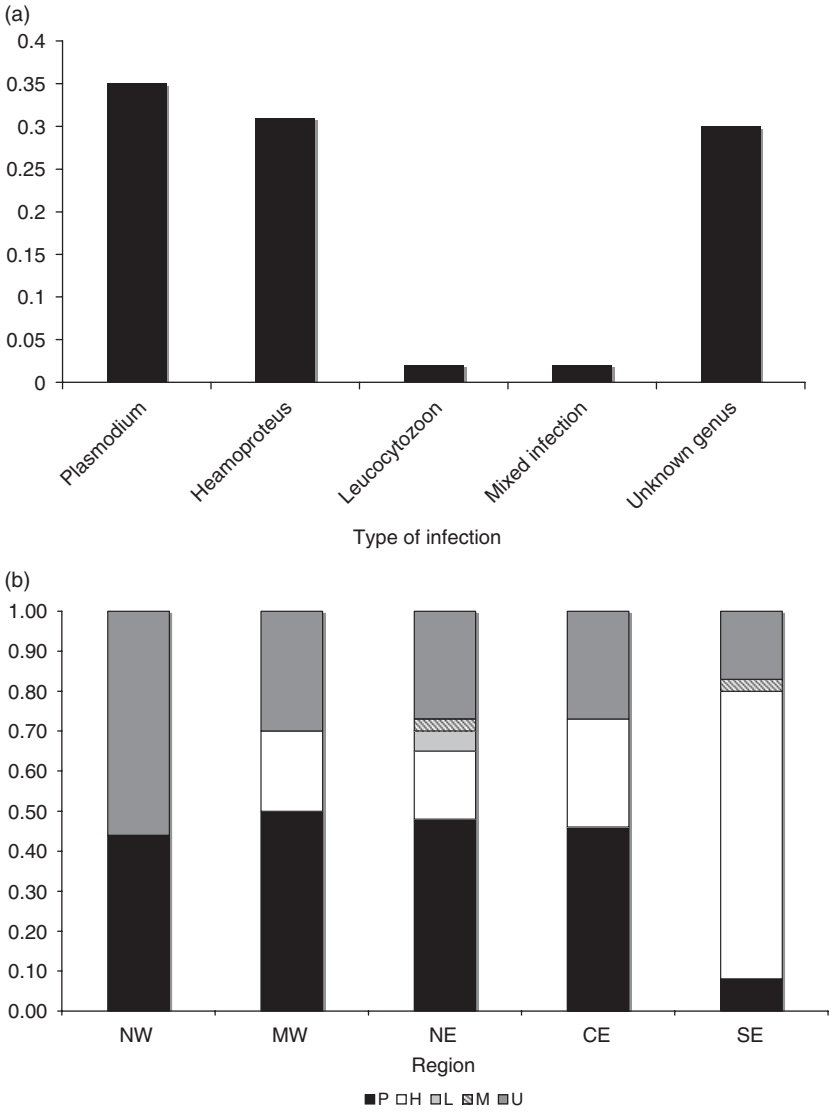

Figure 2 (a) Shows the proportions of different types of haematozoan infection (n=124) across the entire sample of redstarts *Setophaga ruticilla* (n=437). 'Unknown genus' refers to positive infections that could not be identified to genus level due to poor PCR amplification (see Methods). (b) Shows the proportions of different types of infections between regions sampled on the breeding grounds. P, *Plasmodium* spp.; H, *Haemoproteus* spp.; L, *Leucocytozoon* spp.; M, mixed infection of more than one lineage; U, unknown genus.

origins to lineages on the phylogenetic tree and asked: given the sampling, how unlikely were the chances of recovering region-specific clades such as (1H–5H) or (13H–15H)? We performed 100 randomizations and determined regional clustering to be significant if regional homogeneity was recovered in fewer than five trials.

Results

Prevalence of infection

Of 437 redstarts sampled, 124 were infected with haematozoa [prevalence of infection (PoI) = 0.28]. Prevalence differed significantly ($\chi^2 = 23.52$, d.f. = 1, $P = <0.0001$) between samples from the wintering grounds (PoI = 0.03) and breeding grounds (PoI = 0.33). Two infections were recovered in 68 wintering-ground samples, a single *Plasmodium* and a single *Haemoproteus*, neither of which produced fragments of sufficient length for phylogenetic analysis. Both 'mixed' infections of more than one haematozoan lineage, identified by double-peaked sequence, and *Leucocytozoon* spp. were detected in very low frequencies and were not considered further (Fig. 2a). Prevalence of infection was significantly different between breeding-ground geographic regions ($\chi^2 = 17.90$, d.f. = 4, $P = 0.0013$; Fig. 2a; Table 1). Prevalence by type of infection on the breeding grounds was also significantly different between regions (Fisher's exact test $P = <0.0001$; Fig. 2b). In particular, the SE region is different from the rest: prevalence of overall infections was higher in the SE region; and *Haemoproteus* spp. infections were much more common in the SE region while *Plasmodium* spp. infections were much more common in the central and northern regions (see Table 1). Sex and age data were available for the majority of host samples. Males and females were infected with haematozoans in equal proportions, as well as adult (second-year and older) and

hatch-year birds, indicating transmission of parasites occurred on the breeding grounds. We used a sub-sample of birds collected in two breeding seasons, 2002 and 2006, in the same region, Louisiana in the SE, to test for year-to-year temporal effects. There was no difference in prevalence (18 infected of $n = 30$ in 2002, and 17 infected of $n = 37$ in 2006, $t = 1.14$, d.f. = 65, $P = 0.25$), number of lineages detected (five in 2002 and six in 2006, $\chi^2 = 0.091$, d.f. = 1, $P = 0.76$) or the phylogenetic distribution of those lineages ($\chi^2 = 10.3$, d.f. = 9, $P = 0.32$).

Table 1 The prevalence of infection of haematozoa among sampled redstarts *Setophaga ruticilla* in the wintering grounds (bold) and the breeding grounds

Region	*n* sampled	*n* infected	Proportion infected
Caribbean	40	1	0.02
Middle America	25	1	0.04
NW	79	25	0.32
MW	39	10	0.26
NE	136	40	0.29
CE	51	11	0.22
SE	67	36	0.54

Regions correspond to the regions in Fig. 1. Prevalence between regions in the breeding grounds are significantly different.
NW, north-west; MW, middle-west; NE, north-east; CE, central-east; SE, south-east.

Phylogeographical analyses

Of 124 redstarts infected with haematozoa, 59 yielded sequence data of sufficient length for phylogenetic analysis. We recovered 15 *Haemoproteus* haplotypes and 10 *Plasmodium* haplotypes (Fig. 3). Four *Plasmodium* sequences but none of the *Haemoproteus* sequences matched existing sequences on GenBank (see Fig. 3). New sequences have been deposited in GenBank. The maximum-likelihood tree indicated considerable heterogeneity in the distribution of clades between the five geographical regions, most noticeably between the SE region and the central and northern regions. The SE region had two unique *Haemoproteus* clades, consisting of five (1H–5H) and three (13H–15H) haplotypes each, not shared with the rest of the breeding ground. The randomization results indicated that the

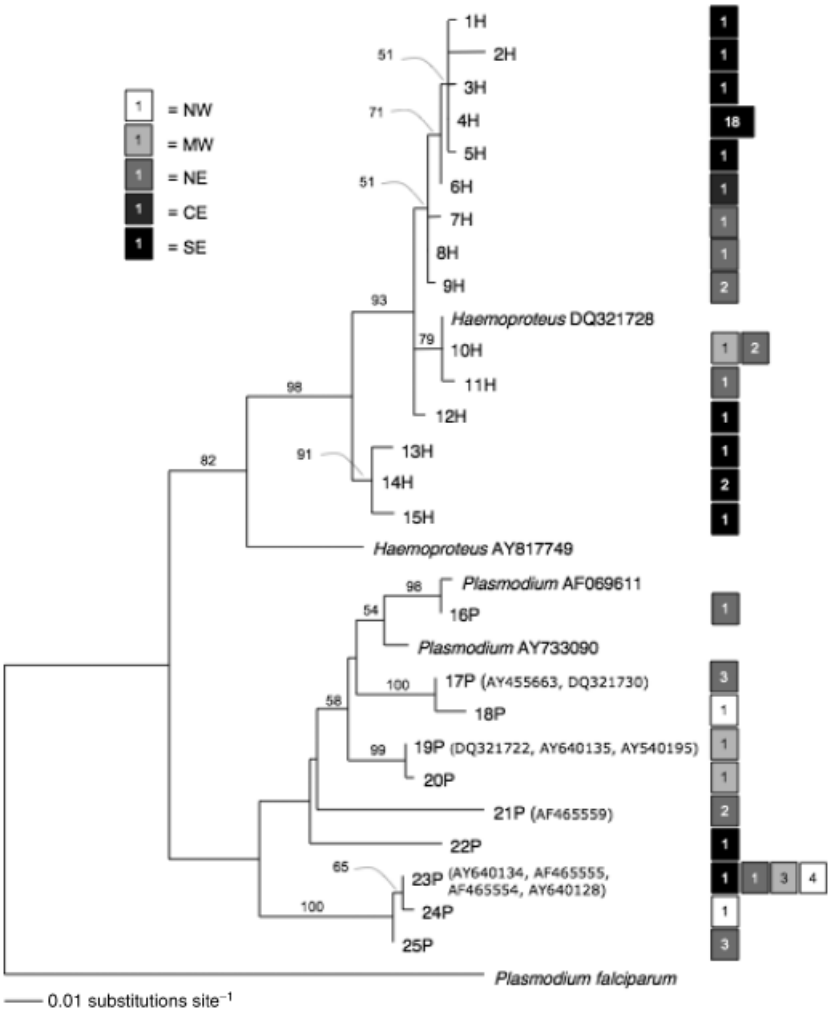

Figure 3 Phylogenetic tree of mitochondrial DNA cyt *b* lineages from haematozoa amplified from redstart *Setophaga ruticilla* hosts sampled on the breeding grounds constructed using maximum likelihood (GTR + G). The distribution of lineages across breeding ground geographic regions is indicated by corresponding coloured squares (shaded black to white), numbers inside the squares indicate the number of individual redstart hosts that the lineage was amplified from. New sequences were deposited in GenBank (Accession numbers: EF187472–EF187496). Where a lineage sequence matched one already catalogued on GenBank, the accession number was recorded in the tree. Bootstrap support (×100 replications) values greater than 50 are found above the relevant branches. The tree was rooted using the mtDNA cyt *b* sequence data of *Plasmodium falciparum*.

Table 2 GenBank accession numbers that matched our recovered sequences for four Plasmodium haplotypes

Haplotype	GenBank accession number	Host species	Range overlap with American redstart *Setophaga ruticilla*?
17P	AY455663	*Seiurus aurocapillus*, Ovenbird	Yes
	DQ321730	*Dendroica caerulescens*, Black-throated blue warbler	Yes
19P	DQ321722	*Dendroica caerulescens*, Black-throated blue warbler	Yes
	AY640135	*Melospiza melodia*, Song sparrow	Yes
	AY540195	*Seiurus aurocapillus*, Ovenbird	Yes
21P	AF465559	*Vireo griseus*, White-eyed vireo	Yes
23P	AY640134	*Dendroica petechia*, Yellow warbler	Yes
	AF465555	*Baelophus bicolor*, Tufted titmouse	Yes
	AF465554	*Dendroica magnolia*, Magnolia warbler	Yes
	AY640128	*Tachycineta bicolor*, Tree swallow	Yes
		Vermivora pinus, Blue-winged warbler	Yes
		Dendroica petechia, Yellow warbler	Yes
		Dendroica pensylvanica, Chestnut-sided warbler	Yes
		Geothylpis trichas, Common yellowthroat	Yes

The host species is the avian host that the particular sequence was recovered from, along with an indication of whether that host species has a range overlap with the redstart on either the breeding or wintering grounds. Exact sampling locations were not available for all matching GenBank sequences.

well-supported clade of *Haemoproteus* haplotypes 13H–15H inclusive was not more likely to be grouped together in a single region than expected by chance [P (single-region grouping) = 0.30]. However, the *Haemoproteus* haplotype clade 1H–5H was much more likely to be found in a single region than expected by chance [P (single-region grouping) <0.01]. There was also an 'eastern' clade of *Haemoproteus*, consisting of samples from the north-east (NE), central-east (CE) and south-east (SE) regions (1H–9H).

A single *Plasmodium* lineage (22P), evolutionarily distant from other *Plasmodium*, was unique to the SE. The single haplotype (23P) that the SE region shared with other regions appears to be a common *Plasmodium* lineage in North America; it was found in four of the five geographic regions and recovered four times on GenBank (see Fig. 3). The NW region was characterized by an absence of *Haemoproteus* infections. All recovered sequences that matched haematozoan sequences deposited in GenBank were recovered from host species whose ranges overlapped that of the redstart, indicating a pool of common *Plasmodium* lineages in some regions of North America that infect multiple species (Table 2).

The Chao2 (S_2^*) estimate of species richness was greater than the number of lineages actually detected for both *Plasmodium* ($S_{obs} = 10$, $S_2^* = 17.2$, SD = 7.1, 95% CI = 11.4–46.4) and *Haemoproteus* ($S_{obs} = 15$, $S_2^* = 32.8$, SD = 12.31, 95% CI = 20.2–75.6). It should be noted that the standard deviation and confidence intervals are large, especially for the *Haemoproteus* lineage estimate. Table 3 shows that for at least three geographic regions on the breeding grounds [NW, middle-west (MW) and NE), we have come close to sampling all expected haematozoa lineages.

Table 3 The difference in expected lineages and actual detected lineages of all genera of haematozoa given sampling effort in each breeding ground region (with the exception of CE)

Region	*n* lineages (S_{obs})	S_2^* mean	95% CI lower limit	95% CI upper limit
NW	**3**	3.83	**3.06**	13.99
MW	**4**	6.50	**4.30**	24.32
NE	**10**	12.35	**10.36**	25.20
CE	1	NA	NA	NA
SE	11	28.37	15.58	76.84

Note that the actual detected lineages are approaching the lower limit of the 95% CI (values marked in bold), indicating our sampling may have found all haematozoa lineages that were present in those regions.
NW, north-west; MW, middle-west; NE, north-east; CE, central-east; SE, south-east; NA, not applicable; CI, confidence interval.

Discussion

Consistent with Webster *et al.*'s (2002) predictions, there appears to be distinct geographic structuring within haematozoan parasite assemblages of the redstart on the North American breeding grounds. This structuring also appears in the prevalence data to a lesser extent. The major difference occurs between the SE portion of the breeding range and the rest of the breeding range. South-eastern redstarts were more likely to be infected by *Haemoproteus* than *Plasmodium* and to carry lineages of *Haemoproteus* unique to the region. Further sampling may reveal the importance of the 'eastern' clade of *Haemoproteus* (1H–9H) in separating eastern and western breeding populations of redstarts. Additionally, NW redstarts do not appear to be infected by *Haemoproteus* as often as *Plasmodium*, and prevalence was lower. Our Chao2 estimates indicated that the number of lineages we detected in the NW, MW and NE regions at least approached the lower boundary of expected numbers of lineages for these regions. It appears that the SE region is distinct among breeding ground populations, and there is

also a possibility of greater east–west structuring between breeding populations of redstarts as elucidated from their parasite assemblages. Other large-scale studies that have attempted to use haematozoa as geographical markers in long-distance migratory bird populations have not been able to show any significant geographical structuring of parasite assemblages (Fallon *et al*., 2006; Durrant *et al*., 2007; Pagenkopp *et al*., in press).

Interestingly, our host–parasite geographical structuring appears to be consistent with patterns of connectivity between redstart breeding and wintering populations inferred from stable isotopes. Using stable-hydrogen isotopes, Norris *et al*. (2006*a*) provided evidence that redstarts exhibit a regional pattern of chain migration in the eastern portion of their range, birds from northerly latitudes were most likely to migrate to northern wintering sites and birds from the southern portion of the breeding range were most likely to migrate to the southern-most wintering sites (Norris *et al*., 2006*a*). Importantly, all birds wintering in Trinidad and Tobago were estimated to have originated from either the SE (93%) or the CE (7%) breeding regions. Our results show that birds of the SE also have a distinct haematozoan assemblage compared with the other regions. Thus, separation of the wintering and breeding populations of the redstart 'host' is reflected in a concordant separation of their parasites. In particular, the SE sample is distinct from the rest of the breeding grounds, carrying a different parasite assemblage, and this distinctness is also indicated from the isotopic data as they winter in isolation from most of the rest of the population. Our results represent a confirmation of the work of Norris *et al*. (2006*a*) for at least the SE population of redstarts. Our results, however, do not equal migratory connectivity. It may be that without corroborating wintering-ground data, this technique is inadequate to determine large-scale migratory connectivity. Transmission of parasites appears to occur on the breeding grounds, as demonstrated by the occurrence of hatch-year birds caught on the breeding grounds with detectable haematozoa, and infections therefore may not be detectable in wintering ground samples. However, these infections have the potential to become chronic, and can persist in host-species for many months (Wolfson, 1941), and still be detected by PCR (K. L. Durrant & R. C. Fleischer, unpubl. data from domestic canaries). Thus, there was a possibility of detecting infections on the wintering grounds that had been transmitted in the previous breeding season.

Patterns of lineage distribution that we were able to detect are likely influenced by local vector communities. Although some vectors potentially remain to be discovered, the major vectors of *Haemoproteus* are biting midges, *Culicoides* (Ceratopogonidae) and Hippoboscidae flies (Atkinson, Forrester & Greiner, 1988; Mullens *et al*., 2006). Atkinson *et al*. (1988) noted a marked difference in the seasonal transmission of *Haemoproteus* between warmer and more temperate regions in Florida. The same effect could be driving the patterns we detected in range-wide distribution of *Haemoproteus* lineages in a host whose breeding range encompasses the warm SE portion of the USA, and more temperate climes found in the species range from Newfoundland to British Columbia. However, it is likely that differences in wintering-ground vector communities also play a role. The large numbers of cryptic haematozoa lineages being uncovered (Bensch *et al*., 2004) means that knowledge of vector species and transmission cycles do not exist for the majority of taxa, and therefore biogeographical information for the vectors of avian haematozoa is scarce.

Our wintering ground sample was not as extensive or large as the breeding ground sample, and prevalence of infection was very low. Regional seasonal fluctuations in haematozoa infection prevalence have been both argued for (Atkinson *et al*., 1988) and against (Waldenström *et al*., 2002, Neotropical example from Fallon *et al*., 2004). Parasite prevalence, in terms of circulating blood-stage parasitemia, may be reduced on the tropical wintering grounds for the redstart, possibly associated with the absence of other physiological stressors promoted by spring migration and breeding activity. Year-to-year temporal effects, as with regional seasonal effects, have been detected in some studies (Bensch & Åkesson, 2003) but not in others, probably the most tightly controlled of these (birds sampled in consecutive years within a few particular days during the breeding season) found no conclusively strong effect of year-to-year temporal fluctuations in the prevalence or lineage presence in blue tits, *Cyanistes caeruleus* (Wood *et al*., 2007). Our own sub-sample of birds collected in Louisiana during the breeding season in 2002 and 2006 showed no significant changes between sampling periods in prevalence, number of lineages detected or lineage distribution, and we consider the possibility of a significant year-to-year temporal effect on our results negligible.

The haematozoan lineage structuring revealed a pattern of SE versus the rest of the breeding range, and a general east-west split in redstart parasite assemblages. This is at odds with recently revealed divergence between the mtDNA sequences of breeding redstarts sampled in Newfoundland and the rest of the breeding population (Colbeck *et al*., in review). The phylogeographic data for the redstart indicate a Pleistocene refugium in Newfoundland. Our parasite data show a differing pattern of structure, representing more contemporaneous processes, but also necessarily complicated by influence from movement of parasite vectors. These two sources of information should be combined when considering the evolutionary and ecological history of the redstart.

Future work on this species should include a wider sampling of individuals caught on the wintering grounds to determine if the SE parasite assemblage is also apparent in the redstart's Trinidad and Tobago wintering grounds (Norris *et al*., 2006*a*). To fully complete the picture, information on local parasite assemblages for sedentary species in wintering and breeding grounds as well as vector information would be invaluable. Determining parasite community structure can only be attempted where there is extensive sampling across the entire range of the host, which highlights the need for cross-disciplinary collaborations in

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delineating migratory connectivity. Establishing broad-scale patterns of lineage distribution and prevalence of parasites are critical for understanding not only migratory connectivity but also epizootic interactions across the host species.

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