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Avian migration and the distribution of malaria parasites in New World passerine birds

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ABSTRACT

Aim Migrating birds transport their parasites, often over long distances, but little is known about the transfer of these parasites to resident hosts in either the wintering or breeding ranges of the migratory host populations. We investigated the haemosporidian parasite faunas of migratory and resident birds to determine connections among distant parasite faunas, plausibly brought about by migratory host populations.

Location Samples were obtained, primarily during or shortly after the local breeding season, throughout the Americas, from the United States through the Caribbean Basin and into northern South America.

Methods Infections were identified by PCR and sequencing of parasite DNA in avian blood samples. The analyses were based on *c.* 4700 infections representing 79 parasite lineages of *Plasmodium* and *Haemoproteus* spp. Geographical connections of lineages between regions in the Americas were compared to those in the Euro-African migration system, where migration distances are longer for many host species and the migrant and resident avifaunas in the wintering areas are phylogenetically more divergent.

Results Haemosporidian lineages exhibited considerable variation in distribution in the Americas, and patterns of distribution differ markedly between the Americas and the Euro-African migration system. In particular, few lineages were recovered from resident species in both temperate and tropical latitudes, particularly in the Euro-African system, in which a large proportion of lineages were restricted to migrants. Parasite lineages in the Euro-African system exhibited considerable phylogenetic conservatism in their distributions, that is, a tendency of related lineages to exhibit similar geographical distributions. In contrast, clades of parasites in the Americas displayed more geographical diversity, with four of 12 clades exhibiting all four of the distribution types representing the combinations of resident and migrant host species in both temperate and tropical latitudes.

Main conclusions Long-distance migrants connect communities of avian haemosporidian parasites in breeding and wintering areas with disparate avifaunas and different vector communities. The degree of parasite lineage sharing between migrants and residents in breeding and wintering areas appears to reflect, to a large degree, the taxonomic similarity of migrants to the resident species in both areas.

Keywords

Haemoproteus*, Haemosporida, *Leucocytozoon*, migratory connections, *Parahaemoproteus*, parasite communities, *Plasmodium

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INTRODUCTION

Parasite dispersal is of concern when it brings the possibility of emerging infectious diseases in humans or in their crops and domesticated animals (Schrag & Wiener, 1995; Daszak *et al.*, 2000; Cleaveland *et al.*, 2001; Friend *et al.*, 2001; Hosseini *et al.*, 2006; Lips *et al.*, 2006; Rachowicz *et al.*, 2006; Robinson *et al.*, 2010; Fuller *et al.*, 2012). Movements of host individuals, including long-distance migration, have extended the geographical ranges of their parasites and pathogens, as in the rapid spread of the introduced West Nile virus throughout North America (Rappole *et al.*, 2000). Migration might also reduce exposure to parasites in either the breeding or non-breeding parts of a host species' distribution, or increase exposure by concentrating individuals at stopover sites or by traversing a wide variety of environments during migration (Hall *et al.*, 2014). However, few studies have examined the general role of avian migration in the distribution of parasite populations (see Valkiūnas, 1993, 2005, pp. 145–163).

Parasites lacking free-living stages are able to disperse only with their hosts and vectors. Many avian parasites potentially could move between temperate and tropical latitudes during the annual migrations of their hosts (Rappole *et al.*, 2000; Zeller & Murgue, 2001; Ricklefs *et al.*, 2005a; Jourdain *et al.*, 2007; Altizer *et al.*, 2011; Møller & Szép, 2011; Fuller *et al.*, 2012). Each year, hundreds of species of birds leave their tropical and subtropical wintering areas for high-latitude summer breeding grounds, returning to lower latitudes at the end of the breeding season (Alerstam *et al.*, 2003; Faaborg *et al.*, 2010). These species are exposed to different parasites in their boreal or temperate breeding areas and in their subtropical or tropical wintering areas, and they potentially could disperse parasites between these areas (Valkiūnas, 1993; Altizer *et al.*, 2011; Levin *et al.*, 2013; Smith & Ramey, 2015; Levin *et al.*, 2016), particularly because birds retain parasite infections through the annual cycle, and often for many years (e.g. Atkinson *et al.*, 2000). The present study addresses the latitudinal distributions of avian haemosporidian parasites (phylum Apicomplexa: order Haemosporida: *Plasmodium*, *Haemoproteus* and *Leucocytozoon*) (Valkiūnas, 2005), which are blood-borne parasites transmitted between their vertebrate hosts by blood-feeding dipteran insects.

Hellgren *et al.* (2007) examined the host and geographical distributions of 259 lineages of haemosporidian parasites in birds of Europe and Africa to determine the degree to which migrants might be involved in the movement of parasites over large geographical distances. A surprising result of that analysis was that only two haemosporidian lineages were recovered from resident host species in both tropical and temperate regions, although eight *Plasmodium*, 18 *Haemoproteus*, and five *Leucocytozoon* lineages were found in migrant host species both on their European breeding grounds and in their tropical or subtropical wintering areas. Presumably, these 31 lineages that infect migrants potentially could be transmitted to local residents in both areas. However, transmission of a parasite between migrants and local residents in both the summer and winter ranges evidently occurs rarely in this system, in spite of the observation that 21 lineages of parasites, or 12.5% of the 168 lineages recovered in the northern breeding distribution of the migrants, were shared between migrants and residents, and 16 lineages of 122 (13.1%), were shared between migrants and residents on the tropical wintering grounds of the migrants (see also Waldenström *et al.*, 2002). Of course, if sharing between migrants and residents occurred at random, one would expect only a small proportion ($0.125 \times 0.131 = 0.016$) to be shared on both the wintering and breeding grounds, assuming that all the parasite–host combinations were recovered; accordingly, 0.016×250 parasite lineages in the Hellgren *et al.* (2007) analysis would result in an expected 4.1 lineages, which is not so different from the observed value of 2.

The sharing of parasites between host species must depend, in part, on compatibility of the parasite with the immune defences of potential hosts (Bonneaud *et al.*, 2006). Primate malarias (*Plasmodium* spp.) show both general host phylogenetic conservatism, that is, the tendency of parasite lineages to infect related hosts, and occasional host shifting across large phylogenetic distances (Garamszegi, 2009). Although lineages in some clades of haemosporidian parasites infect only a narrow range of closely related avian host species (Pérez-Tris *et al.*, 2007; Jenkins *et al.*, 2012; Ricklefs *et al.*, 2014), much host shifting appears to occur nearly at random among locally available avian host species in both temperate and tropical regions, even at the host taxonomic family level (Bensch *et al.*, 2000; Ricklefs & Fallon, 2002; Ricklefs *et al.*, 2014). This would imply that parasite lineages should be easily shared between resident and migrant hosts within a region. However, tropical resident hosts, temperate resident hosts and migratory hosts are, to a large extent, distinct phylogenetically. Accordingly, parasites might fail to undergo complete gametogony (i.e. formation of male and female (micro- and macro-) gametocytes in the bloodstream), and thus be incapable of local transmission, in resident host species in one or both regions, although they would be recorded as infections. Furthermore, little is known about the potential of local vectors to transmit particular parasite lineages in both temperate and tropical areas (cf. Gager *et al.*, 2008; Hellgren *et al.*, 2008; Medeiros *et al.*, 2013; Valkiūnas *et al.*, 2015), although many lineages of all three parasite genera infect resident species in both regions, demonstrating local transmission. Finally, resident and migrant birds often are separated locally by habitat (Keast & Morton, 1980; Hutto, 1985; Sherry & Holmes, 1996; Sanderson *et al.*, 2006), which might reduce the potential for disease transmission.

Here, we analyse the distributions of haemosporidian parasite lineages (*Plasmodium* and *Haemoproteus*) among residents and migrants in eastern North America and in the tropical wintering grounds for migrants in the West Indies and northern South America. This Western Hemisphere

migration system differs from that between Europe and Africa in that migration distances generally are shorter (Rappole, 2013), especially to the islands of the Greater Antilles and to Central America. Moreover, migrants are more likely to encounter related resident species in the breeding and wintering areas in the Americas than in Europe and Africa (Newton, 2008; Cox, 2010; Rappole, 2013), perhaps increasing the probability of transmission between migrant and resident hosts.

Our analysis is based on haemosporidian infections detected by PCR, and identified by DNA sequencing, in extensive collections of blood samples, primarily from passerine birds (order Passeriformes) in the eastern United States, the West Indies, Central America (southern Mexico and Panama) and tropical South America, especially northern Venezuela, Amazonian Ecuador and the Brazilian Cerrado. Our primary goal in this analysis was to compare the Euro-African and the American migration systems with respect to the degree to which seasonal, population-level, long-distance movements connect the parasite assemblages of geographically and ecologically distant regions. Consistent with Hellgren *et al.* (2007), we found that, although lineage diversity in our tropical and temperate samples was similar, few parasite lineages were recovered from both temperate and tropical resident hosts. However, the Western Hemisphere system differs from the Euro-African system in the absence of lineages restricted to either resident or migrant host species in the temperate zone, as well as the absence of lineages of parasites recovered uniquely from individuals of migrant species in the tropics. Overall, migration provides a stronger connection between the parasite faunas of resident birds in the Americas compared to those in Europe and Africa.

MATERIALS AND METHODS

Field and laboratory methods

The analyses are based on collections of blood samples obtained at locations in eastern North America (Alabama, Connecticut, Illinois, Indiana, Louisiana, Michigan, Missouri, Pennsylvania and Tennessee), the Caribbean Basin, including the Yucatan Peninsula and all the major islands of the West Indies except Cuba, and tropical South America (Brazil, Ecuador, Trinidad and northern Venezuela), including central Panama (see Appendix S1 in Supporting Information for details of collection sites and number of samples). Most of the samples were collected during or shortly after the breeding season in each locality. However, extensive samples from the Guanica Forest in Puerto Rico exhibited little variation in parasite communities at different times during the year (Fallon *et al.*, 2004). Svensson-Coelho *et al.* (2013) found prevalence to be homogeneous among years in six of seven common host species analysed in Tiputini, Ecuador. Few temperate-zone breeders were sampled on their wintering grounds, with the exception of winter samples from Puerto Rico and the Dominican Republic (Hispaniola), where most lineages of parasites infecting wintering birds also were recovered on the breeding grounds.

Field and laboratory methods are described in detail in Ricklefs *et al.* (2005b), Fallon *et al.* (2005), Fallon & Ricklefs (2008), Svensson & Ricklefs (2009), Latta & Ricklefs (2010) and Fecchio *et al.* (2013). Briefly, all birds were caught by mist net under permit from local governments and appropriate IACUC protocols. A small sample of blood was obtained from each individual by venipuncture from a vein in the wing and stored in PureGene or Longmire's lysis buffer (Longmire *et al.*, 1997), or in absolute ethanol, after which the bird was released unharmed. Blood samples were brought, under the appropriate USFWS and APHIS permits, from the field to the laboratory, where DNA was extracted. We screened each DNA sample for the presence of *Plasmodium* or *Haemoproteus* with primers that amplify a 154 base pair segment of the mitochondrial 16S rRNA gene (Fallon *et al.*, 2003; Bell *et al.*, 2015). For all positive samples, we sequenced a *c.* 500 base pair portion of the cytochrome *b* gene to establish the lineage identity of each infection (see Ricklefs *et al.*, 2005b; Svensson-Coelho *et al.*, 2013).

Phylogenetic relationships among the sequences were analysed by maximum likelihood using the RAxML blackbox (http: embnet.vital-it.ch/raxml-bb/) (Stamatakis, 2006; Stamatakis *et al.*, 2008) with the default general time reversal model of nucleotide substitution with 100 bootstrap iterations, and visualized in Figtree, written by Andrew Rambaut (http://tree.bio.ed.ac.uk/software/figtree/).

Lineage designation

Although Hellgren *et al.* (2007) designated each unique sequence as a different lineage, here we allow a degree of variation in cytochrome *b* sequences within lineages, as long as host and geographical distributions are consistent (see, e.g. Svensson-Coelho *et al.*, 2013). This difference in lineage designation likely explains part of the approximately threefold difference in number of lineages recognized in the two studies. Recognizing finer distinctions between lineages reduces the probability of matching two parasites to the same lineage and possibly accounts for some of the lower migratory connectivity reported in Hellgren *et al.*'s (2007) study compared to the present analysis. Although species limits pose challenges in haemosporidian parasites (Outlaw & Ricklefs, 2014), genetic differences of the magnitude recognized in this study are consistent with significant linkage disequilibrium in other studies using multiple independent markers (e.g. Bensch *et al.*, 2004; Nilsson *et al.*, 2016), indicating non-mixing parasite populations. An important caveat in any DNA analysis of haemosporidian infections is that positive samples do not necessarily represent development of gametocytes, which is required for successful transmission by dipteran vectors (Valkiūnas, 2005). We have not verified the presence of gametocytes by microscopic examination of blood smears in this study for every combination of host species and parasite lineage, but close examination of

hundreds of slides known to be infected by common lineages in a particular host has, as a rule, shown gametocytes to be present.

Biogeographical analysis

Based on a variety of field guides, handbooks and web sites, each host species was classified as either non-migratory, in which case it is resident in either the temperate region, the tropical region or (rarely) both; or, migratory, in which case the host could be sampled in the temperate portion of its distribution, where it breeds, in the tropical region, where it winters, or both. There are 15 combinations of presence or absence in each of these four categories (temperate, tropical, migratory and non-migratory; not counting 'none of these') and we assigned each of the parasite lineages to one of these categories, depending on the type of host and the sample locations of the parasites recovered from migratory species, following Hellgren *et al.* (2007). Unlike their protocols, however, our laboratory methods generally do not amplify infections of *Leucocytozoon*, which are not included here. Note, however, that in the Hellgren *et al.* (2007) study, the distribution of *Leucocytozoon* lineages among temperate and tropical locations and among migrant and resident species matched that of *Plasmodium* and *Haemoproteus* closely (see Table 1).

Table 1 Number of lineages of haemosporidian parasites exhibiting each combination of location and status as a migrant or resident, in this study in the Western Hemisphere and that of Hellgren *et al.* (2007) in Europe and Africa. Pluses (+) in the columns at left indicate the unique distribution pattern of the parasites in each row.

Tropical		Temperate		This study		Hellgren *et al.* (2007)			
Res	Migr	Migr	Res	Pla	Hae	Pla	Hae	Leu	Total
+	+	+	+	2		1			3
+	+	+		3	5	4	6		18
+	+		+						0
+	+				2	2	1	2	7
	+	+	+	4	1	2	5	1	13
	+	+			1	1	7	4	13
	+		+						0
	+					5	10	6	21
+		+	+	3	5				8
+		+		3	3	6	2		14
+			+	1	2		1		4
+				14	16	13	28	15	86
		+	+	3	1	3	5	4	16
		+			1	21	28	14	64
			+	4	5	6	30	17	62
Total				37	42	64	123	63	329
Total per study					79			250	
Temperate resident lineages				17	14	12	41	22	
Tropical resident lineages				26	33	26	38	17	

Migr = migrant species; Res = resident (i.e. non-migrant) species; Pla = *Plasmodium*; Hae = *Haemoproteus*; Leu = *Leucocytozoon*.

We analysed the distributions of parasite lineages among host locations (tropical versus temperate) and the status of hosts as migrants or residents, by contingency analyses. Details of statistical tests are provided in the results section. We did not test for prevalence variation among our categories, as this was beyond the scope of our study. Also, we did not account for host phylogenetic relationship in these analyses because we have found that host switching by haemosporidian lineages occurs largely at random with respect to the phylogenetic relationship of host species within a region (Ricklefs *et al.*, 2014). Also, because large numbers of both resident and migratory bird species were included in our samples from both tropical and temperate locations, we did not correct for potential sampling biases in the analyses. However, the majority of hosts sampled in the temperate region were migrants, reflecting the makeup of the North American passerine avifauna; hosts sampled in the tropics were relatively evenly divided between residents and migrants in Central America and the Greater Antilles, but migrants were infrequent in the Lesser Antilles and in South America. Because we have not quantified the distributions of host species in our analyses, we limit comparisons between studies to general patterns.

RESULTS

We assembled a database of haemosporidian infections of the genera *Plasmodium* (37 lineages and 2022 host individuals) and *Haemoproteus* subgenus *Parahaemoproteus* (42 lineages and 1982 host individuals), representing lineages recovered from at least eight host individuals, a number that we chose to balance sample size per parasite lineage against number of lineages included in the analysis (see Appendix S2, for a list of lineages included in this analysis). Host individuals were placed in four categories (the combinations of temperate versus tropical and migrant versus resident) and a sample size of twice this number seemed like a minimum, although 62.6% of the 219 lineages in our database were represented by fewer cases. We did not include two lineages of *Haemoproteus* subgenus *Haemoproteus*, which are practically restricted to doves and pigeons (Columbidae).

Distributions of parasite lineages with respect to location and host status as migrant or resident are compared between this study and that of Hellgren *et al.* (2007) in Table 1 and Fig. 1. These distributions exhibit considerable heterogeneity with respect to different combinations of location and migrant status, as well as differences between the two studies. First, only three lineages, all *Plasmodium*, were found in both migrant and resident host species in both regions. Indeed, few lineages were recovered from resident species in both tropical and temperate regions (15 of 329, evenly divided between *Plasmodium* and *Haemoproteus* but including no lineages of *Leucocytozoon*), particularly in the Euro-African system (only two lineages) (Table 2). A contingency test of the association between the occurrence of lineages in temperate and tropical resident species ($G = 56.2$, d.f. = 1, $P < 0.0001$)

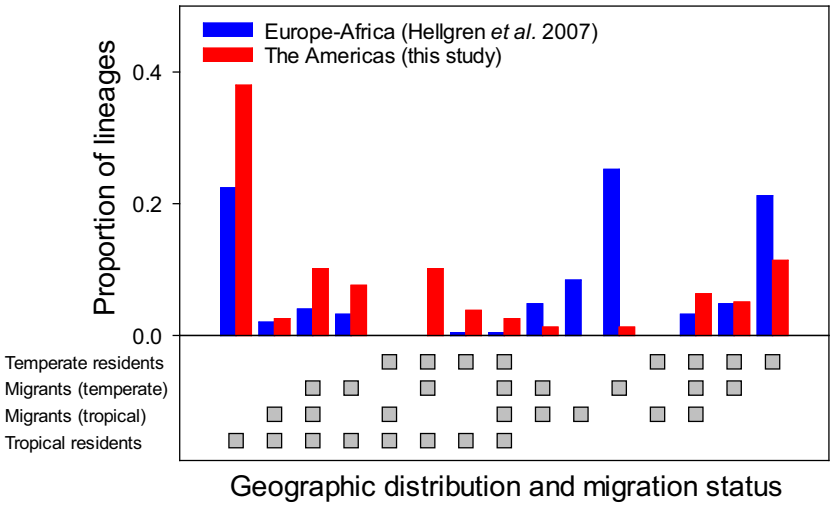

Figure 1 Proportions of lineages (*Plasmodium*, *Haemoproteus* and *Leucocytozoon* combined) identified in the Euro-African (250 lineages) and American (79 lineages) migration systems associated with each combination of host species distribution, that is, presence in temperate and tropical localities, as well as the distinction of resident versus migrant, host species, as indicated by the symbols below the bar chart.

Table 2 Number of haemosporidian lineages recovered from resident species of hosts in temperate and tropical latitudes across both migration systems. Thirteen of the 15 lineages recovered from resident hosts in both the tropics and temperate regions were from the Western Hemisphere.

		Temperate resident	
		Yes	No
Tropical resident	Yes	15	125
	No	91	98

emphasizes the lack of parasite lineages occurring in both. Compared to the migration system in the Americas, avian haemosporidian lineages in Europe and Africa tend to be more diverse in long-distance migrants, which are more frequently sampled in Europe, but these lineages rarely infect resident species in Europe (see Table 1, Temperate migrants).

Details of the host distributions of the 13 Western Hemisphere parasite lineages recovered from both tropical and temperate residents (see Appendix S3) reveal a variety of patterns in host occurrence and relative frequency in tropical and temperate regions, without a consistent pattern. Although many of these lineages were frequent in our samples overall, presence in a particular region or in a particular host species or higher taxon often depended on a small number of host records.

Some additional categories of parasite lineage distribution appear to be uncommon in both the New World and Old World systems. In particular, parasites restricted to resident species in the temperate region but to long-distant migrants in the tropics are underrepresented; the few that do occur also were found in migrant hosts in the temperate region. This suggests that the parasites of temperate residents and long-distance migrants might be drawn from different clades of parasites (Waldenström *et al.*, 2002). We examined this possibility by considering the distributions of parasite lineages in a number of parasite evolutionary clades. Phylogenetic reconstruction of relationships among *Plasmodium* and *Haemoproteus* lineages (Fig. 2) permitted examination of the distributions of closely related parasite lineages with respect to the geographical distribution and migration status of their hosts (Table 3).

In the New World, parasite lineages in most clades were recovered from both temperate and tropical regions (10 of 12 clades), from both resident and migratory host species (11 of 12 clades), and, in four cases, from all four combinations of region and migration status (Table 3). This suggests substantial evolutionary lability of region and migration status within clades of closely related parasite lineages, which is consistent with their general host-taxon lability (Ricklefs *et al.*, 2014). Indeed, the host distribution of parasite lineages might be broader than is evident from Table 3 if some lineages do not circulate in the peripheral blood at certain times of year and were not sampled.

DISCUSSION

Our survey of haemosporidian parasites (*Plasmodium* and *Haemoproteus*) in birds of the Americas confirms the heterogeneous distribution of parasite lineages among migrants and residents both on the temperate breeding grounds and in tropical wintering areas, but with apparent differences between the Euro-African and American migration systems. In particular, more parasite lineages are restricted to either migrants or residents, but not both, in the Euro-African sample, which also lacks parasites that occur in resident host species in both temperate and tropical latitudes. This might reflect a greater taxonomic difference between migrants and residents in both breeding and wintering areas in the Euro-African system, compared to the Americas, where most of the more species-rich taxa of migrants are represented in the resident tropical avifaunas of the West Indies, Central America, and northern South America. In the Amazon Basin, however, the passerine avifauna is dominated by suboscine

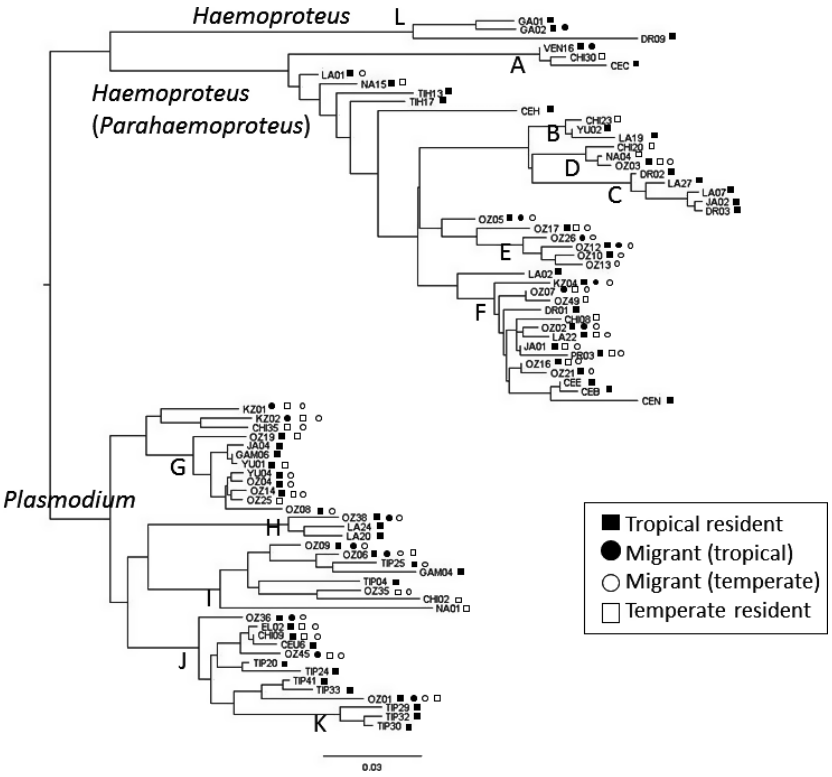

Figure 2 Phylogenetic reconstruction of the 79 haemosporidian lineages analysed here based on cytochrome *b* DNA sequence (see also Ricklefs *et al.*, 2014). Sequences for the lineages are deposited in GenBank. Twelve clades with high bootstrap support (> 80%) are identified by the letters A through L. The symbols show the distribution of each parasite lineage among resident and migrant host species in temperate and tropical regions. The scale bar represents number of nucleotide substitutions per site.

Table 3 Distribution of the hosts of parasite lineages within clades identified in Fig. 2, with respect to the region and the migrant/resident status of the hosts from which they were obtained.

				Tropical hosts*		Temperate hosts*	
Genus	Node	Support	Lineages ≥7 individuals	Resident	Migrant	Resident	Migrant
Hae	A	100	3	2	1		1
Hae	B	99	3	7			2
Hae	C	100	5	5			1
Hae	D	100	3	1		1	3
Hae	E	86	4	2	2	4	
Hae	F	84	14	11	4	8	6
Pla	G	86	8	7	1	5	4
Pla	H	100	3	3	1	1	
Pla	I	99	8	5	2	4	4
Pla	J	87	13	12	3	5	4
Pla	K	100	3	3			
Hae	L	100	3	3	1		

Hae refers to *Haemoproteus* (subgenus *Parahaemoproteus*, nodes A–F; subgenus *Haemoproteus*, node L) and Pla refers to *Plasmodium*. Support is the percentage of bootstrap replicates recovering that clade in maximum likelihood reconstructions of the phylogeny.
*Several lineages are represented in more than one host distribution category.

passerines, and the parasite connections between migrants and residents are more complex; moreover, few North American migrants are distributed so far south during the non-breeding season.

At the sampling location in Amazonian Ecuador, we recorded 21 malaria parasite infections belonging to lineages first described from the Ozarks of southern Missouri. Eleven of these infections, representing lineages OZ01, OZ03, OZ06 and OZ21 (see Appendix S2), were found in suboscine passerines in Ecuador but are typical of emberizids and other oscine passerines in North America, that is, spanning one of the basal nodes in the phylogeny of Passeriformes. Representing more conservative host associations, lineage OZ04 was restricted to emberizids in North America and was recovered from six emberizid host species in Ecuador, primarily tanagers. Lineages OZ09 and OZ36 have been recovered only from vireos in both regions. Nonetheless, half of the parasites of lineages shared between Ecuador and North

America represented substantial host taxonomic shifts between the regions.

One of the most remarkable differences in haemosporidian distributions, emphasized by Hellgren *et al.* (2007), is that only two lineages (out of 250 of all three parasite genera) were found in both temperate and tropical resident birds in their study, compared to 13 of 79 lineages of *Plasmodium* and *Haemoproteus* in this study ($G_{adj} = 26.7$, $P < 0.0001$). Among parasite lineages found in migrants in tropical regions, Hellgren *et al.* (2007) recovered only 31 of 57 in migrants in temperate regions, compared to 16 of 18 in this study ($G_{adj} = 7.7$, $P = 0.005$). One potential cause of this difference between the results for the Euro-African and American migration systems is the different criteria for defining lineages in each system. In general, single nucleotide differences define lineages in the Euro-African system (Bensch *et al.*, 2009), whereas we have allowed for more local genetic diversity within lineages in our analyses of Western Hemisphere haemosporidians (e.g. Ricklefs *et al.*, 2005b; Svensson-Coelho *et al.*, 2013). It is also important to recognize that the presence of a lineage in a particular host does not necessarily indicate a competent host; abortive development and failure to form gametocytes should be considered because this would represent a dead end with respect to parasite transmission. Based on the available data, we cannot know how many of the lineages are reported from competent hosts. Future studies should include microscopic examination of blood smears to establish the presence of gametocytes.

Hellgren *et al.* (2007) suggested that lineages in the parasite genera *Haemoproteus* and *Leucocytozoon* tend to be restricted to a single resident bird fauna, in either the tropical or the temperate region, whereas *Plasmodium* parasites appear to be spread more freely between the avifaunas of the two continents. If this were the case, the relative frequencies of *Plasmodium* and *Haemoproteus* lineages among the geographical distribution classes in Table 1 and Fig. 2 should differ. In the Euro-African data, among lineages recovered in the temperate breeding areas, 38 *Plasmodium* lineages were recovered from migratory species and only 12 from residents, compared to a more even distribution in *Haemoproteus* (53 versus 41; $G_{adj} = 5.5$, $P = 0.02$). Even without taking details of sampling into account, the difference is small. In the American study, parasites recovered in the temperate region revealed no heterogeneity in the proportion of lineages restricted to migrants in *Plasmodium* (18 vs. 17 restricted to residents) compared to *Haemoproteus* (17 vs. 14) ($G_{adj} = 0.8$, d.f. = 1, $P = 0.8$, NS).

Turning this argument around, we note that in the Euro-African system only 9% of *Plasmodium* lineages were restricted to temperate-zone residents, compared to 24% (*Haemoproteus*) and 27% (*Leucocytozoon*) ($G_{adj} = 8.1$, $P = 0.016$), which gives the appearance of a stronger association of *Plasmodium* lineages with migratory species. In the American system, these percentages are 11% and 12% for *Plasmodium* and *Haemoproteus*, respectively, showing no tendency of *Haemoproteus* to specialize on temperate residents. Indeed, migrant and resident species of hosts occur together broadly across habitats, particularly in the West Indies and Central America. In addition, lineages of both genera appear to switch readily among a wide variety of hosts in conjunction with species formation (Ricklefs *et al.*, 2014) (also see Table 3). Moreover, specialization on a narrow range of dipteran primary hosts (i.e. vectors) does not seem to restrict the potential for infecting (or, at least, biting) a wide range of avian secondary hosts (Gager *et al.*, 2008; Medeiros *et al.*, 2013).

Looking more broadly at the distribution classes of *Plasmodium* and *Haemoproteus* lineages in the American data, a contingency test of these frequencies, leaving out four distribution classes with 0 entries for one or the other of the genera (six lineages altogether), was not significant ($G_{adj} = 4.1$, d.f. = 7, $P = 0.77$). The same test applied to the Euro-African data (two distribution categories with two lineages not included) also revealed non-significant heterogeneity in the distribution of these two genera ($G_{adj} = 15.3$, d.f. = 9, $P = 0.08$). Thus, *Plasmodium* does not appear to exhibit unique distribution patterns consistent with facilitated migratory connections between the breeding and wintering areas; among lineages of *Leucocytozoon* parasites, the only deficit compared to *Plasmodium* and *Haemoproteus* in the Euro-African data was the absence of lineages found both in tropical residents and in migrant hosts during the breeding season.

The only other detailed analysis of the distribution of parasites in an avian migration system is that of Jenkins *et al.* (2012), who examined the host distribution of *Leucocytozoon* parasites among hosts and with respect to the geography of migration in the Euro-African system. They showed significant cophylogenetic diversification between *Leucocytozoon* lineages and their avian hosts, in contrast to the absence of evidence for frequent parallel splitting of parasite and host lineages in *Plasmodium* and *Haemoproteus* (Bensch *et al.*, 2000; Ricklefs & Fallon, 2002; Ricklefs *et al.*, 2004; Galen & Witt, 2014; Ricklefs *et al.*, 2014). Jenkins *et al.*'s evidence for cophylogeny depended, in large part, on the phylogenetic localization of several specialized parasite lineages on species of grouse (Tetraonidae), with no other close host relatives in the analysis, two species of tits (Paridae; *Parus major* and *Cyanistes caeruleus*) in Europe, and a number of non-migratory ploceid finches in the tropics. The American data reveal a number of strong associations between vireos (Vireonidae) and their parasites, but few others.

Jenkins *et al.* (2012) also suggested that migratory bird species harbour more phylogenetic diversity of parasite lineages compared to resident species. However, this result is not apparent in the data from either the analysis of Hellgren *et al.* (2007) or from the present analysis. In the Euro-African system, temperate migrants do harbour marginally more parasite lineages (*Plasmodium*+*Haemoproteus* = 91) than temperate residents (*Plasmodium*+*Haemoproteus* = 63) ($G_{adj} = 2.6$, d.f. = 1, $P = 0.11$), but the numbers are

statistically indistinguishable in the Americas (54 vs. 50) ($G_{adj} = 0.12$, d.f. = 1, $P = 0.73$). The results for the two studies also do not differ from each other ($G_{adj} = 0.07$, d.f. = 1, $P = 0.41$). Moreover, the Jenkins *et al.* (2012) study was limited to *Leucocytozoon*, for which Hellgren *et al.* (2007) found roughly equal numbers of lineages restricted to resident (22) and migrant (23) hosts, with five of these infecting both. These analyses do not take into account the number of potential host species that are either residents or migrants, although migrant species outnumber temperate resident species in both migration systems.

Migrants clearly are important agents of distribution of blood parasites across long distances. Migration also presumably increases the exposure of avian populations and individuals to a greater variety of pathogens, with implications for the evolutionary optimization of the host immune system (Møller & Erritzøe, 1998) and the host life history more generally (Hall *et al.*, 2014). Migration might also allow host populations to escape parasites under some circumstances (Altizer *et al.*, 2011). The role of migrants in dispersing haemosporidian lineages between temperate and tropical regions would appear to be magnified in the Americas, perhaps by the relatively short migration distances of many species that winter in Central America and the Caribbean, and by the taxonomic relatedness of a large proportion of the temperate and tropical avifauna. The similar parasite faunas of New World migrants and residents must also reflect, in part, the similar ecological distributions of hosts and migrants, resulting in exposure to the same vectors. However, the general implications of migration for the exposure of potential hosts to infection by one or more pathogens are not well understood. Almost half the parasite lineages recognized in the two studies were restricted to resident species in temperate or tropical areas. Whether lineages that infected migrants, most of which were shared with residents in either the wintering or breeding areas, were ancestral in migrant or resident species is difficult to know. The observation that 38% (96/250) of the Euro-African lineages were restricted to migrants, compared to 3% (2/79) in the American migration system, suggests greater spatial partitioning of the Euro-African system, possibly due to the greater taxonomic partitioning of migrants and residents, but also to sparser sampling of resident species in Africa. Reduced spatial partitioning of parasite lineages within the American migration system would suggest a greater potential of migrants to spread diseases compared to the Euro-African migration system.

Migrant birds potentially can spread pathogens over vast distances, but the probability of newly emerging diseases brought by migrants depends in part on the presence of suitable vectors and the compatibility of disease organisms with both migrant and local resident host species. Because of the low prevalence and host specialization of many parasite lineages, the contribution of migrants to the development of local parasite communities can be assessed adequately only by thorough sampling over time, space and host taxa, including application of parasite-genus-specific primers to help recognize and identify mixed infections, and adoption of uniform criteria for distinguishing parasite lineages. Comparisons between studies will additionally benefit by standardization of sampling and analyses. Neither of these standards was fully achieved in the present comparative analysis, and the underlying causes of apparent differences between the American and Euro-African migration systems cannot be completely resolved at this point.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Samples of birds and haemosporidians.
Appendix S2 Distributions of parasite lineages.
Appendix S3 Details of haemosporidian lineage distributions.

BIOSKETCH

Robert E. Ricklefs is a Professor of Biology at the University of Missouri-St. Louis (UMSL), with interests in the structure and coevolutionary dynamics of populations and ecological communities. **Matthew Medeiros**, **Vincenzo Ellis**, **Leticia Soares** and **Maria Svensson-Coelho** are former doctoral students in the Ricklefs lab; **Alan Fecchio**, **Diana Outlaw** and **Steven Latta** are former postdoctoral fellows; and the other authors are collaborators on avian haemosporidian parasites.

Author contributions: R.E.R. conceived the idea, based on a previous analysis by O.H.; all the authors were involved in collecting samples in the field; M.M., V.E., M.S-C., L.S. and D.O. carried out lab work; R.E.R. compiled and analysed the data; R.E.R. wrote the initial draft of the paper with contributions from all the authors, who read and approved the final manuscript.

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