Host Specialization and Geographic Localization of Avian Malaria Parasites: A Regional Analysis in the Lesser Antilles

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abstract: We recovered 26 genetically distinct avian malaria parasite lineages, based on cytochrome *b* sequences, from a broad survey of terrestrial avifauna of the Lesser Antilles. Here we describe their distributions across host species within a regional biogeographic context. Most parasite lineages were recovered from a few closely related host species. Specialization on one host species and distribution across many hosts were both rare. Geographic patterns of parasite lineages indicated limited dispersal and frequent local extinction. The central islands of the archipelago share similar parasite lineages and patterns of infection. However, the peripheral islands harbor well-differentiated parasite communities, indicating long periods of isolation. Nonetheless, 20 of 26 parasite lineages were recovered from at least one of three other geographic regions, the Greater Antilles, North America, and South America, suggesting rapid dispersal relative to rate of differentiation. Six parasite lineages were restricted to the Lesser Antilles, primarily to endemic host species. Host differences between populations of the same parasite lineage suggest that host preference may evolve more rapidly than mitochondrial gene sequences. Taken together, distributions of avian malarial parasites reveal evidence of coevolution, host switching, extinction, and periodic recolonization events resulting in ecologically dynamic as well as evolutionarily stable patterns of infection.

Keywords: avian malaria, host, parasite, specialization, biogeography, dispersal.

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Malaria parasites (*Haemoproteus* and *Plasmodium*) are globally distributed among birds, infecting many species (Atkinson and Van Riper 1991). Prevalence based on reading blood smears varies between 10% and 30% in regional surveys, although recent polymerase chain reaction (PCR)–based studies suggest that avian malaria may be even more common than previously believed (Richard et al. 2002; Fallon et al. 2003*a*). The widespread geographic distribution of avian malaria parasites and their broad range of host species make them excellent models for exploring the ecological and evolutionary dynamics of host-parasite associations.

Taxonomic distinctions among avian malarial parasites, as well as their geographic and host distributions, have been based on microscopic examination of blood smears. Classical taxonomic practice assumed a high degree of host specificity (Bennett and Peirce 1988; Bennett et al. 1994), though some described species of *Plasmodium* are believed to infect hosts from various avian families on multiple continents (Bennett et al. 1993). Recent molecular analyses of avian malaria parasites based primarily on mitochondrial sequences have revealed a wealth of genetic diversity among parasite lineages that is not apparent in their morphology (Bensch et al. 2000; Perkins and Schall 2002; Ricklefs and Fallon 2002). The distributions of these genetically distinct avian malarial blood parasites are virtually unknown. Molecular phylogenetic studies to date have compared parasite lineages across distantly related hosts, such as humans, rodents, and birds (Waters et al. 1993; Escalante and Ayala 1994; McCutchan et al. 1996; Escalante et al. 1998), among small groups of closely related species (Bensch et al. 2000) or between distant geographic regions (Ricklefs and Fallon 2002). Comprehensive phylogenetic and biogeographic studies within a regional host fauna are now needed to assess community-level host-parasite associations in avian malaria, including the extent of host

specialization and the geographic localization of individual parasite lineages.

The Lesser Antillean archipelago is well suited for studying avian blood parasites because the historical development of the avifauna (i.e., potential host species) has been well documented in an extensive regional phylogeographic analysis (Ricklefs and Bermingham 2001, 2002). In addition, these islands are close enough to continental sources of host and parasite populations to harbor diverse communities yet isolated enough for the evolution of endemic populations (fig. 1). Sequence-based phylogenetic analyses of island bird populations reveal a broad range of differentiation and endemism within the Lesser Antilles (Seutin et al. 1993; Lovette et al. 1999*a*, 1999*b*; Ricklefs and Bermingham 1999, 2001; Hunt et al. 2001). The varying distributions, ages, and evolutionary histories of the host populations within the Lesser Antillean archipelago provide unique opportunities for exploring the development of the parasite community that infects these birds.

Recent studies indicate that the Lesser Antilles harbor a diverse malaria parasite community with varying geographic and host distributions, including extensive host sharing (Apanius et al. 2000; Fallon et al. 2003*b*). Geographic structuring of parasite lineages also suggests that dispersal could be constrained. In addition, a separate study of the temporal stability of avian malaria parasite communities suggested a dynamic system, with colonization and extinction events driven by a combination of host-specific immune responses, competition between lineages, and stochastic change (Fallon et al. 2004). As a result, parasite lineages, like their hosts, appear to exhibit diverse distributions and histories in the region. However, whether these patterns reflect histories similar to their avian host populations or the geography of the region, for example, can be assessed only by investigating parasite lineages across the entire archipelago and neighboring continental localities.

Here we explore the host and geographic distributions of avian malaria parasites in the Lesser Antilles within a broad regional context. Specifically, we assess whether variation in the prevalence of parasite infection is related to ecological and evolutionary traits of their host species. We also use a host breadth index to quantify the extent to which individual parasite lineages specialize among the available host species. With this index, we then test whether host specialization is related to other aspects of a parasite lineage's distribution, including its abundance and geographic range.

In addition, we describe the degree of geographic continuity, or structuring, of parasite lineages between discrete island and mainland locations. We use this regional-biogeographic analysis to evaluate patterns of dispersal, extinction, endemism, and the relationship of the Lesser Antillean parasite fauna to that of source areas in other regions. Together these patterns allow us to assess general characteristics of the ecological and evolutionary forces that determine host specialization and geographic localization of avian malarial parasite lineages and thus the shaping of a regional parasite fauna.

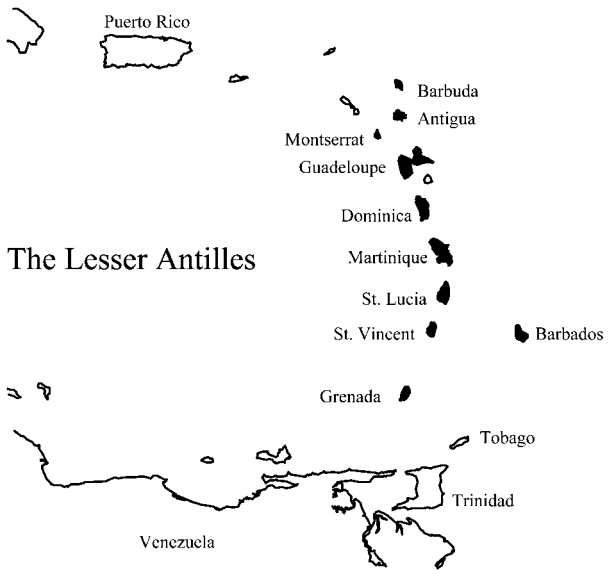

Figure 1: Map of the Lesser Antillean archipelago. Sampled islands are solid black areas.

Material and Methods

Sampling

Blood samples were collected from nearly 2,000 individual birds from 10 islands in the Lesser Antilles (table A1 in the appendix in the online edition of the *American Naturalist*). Sampling effort and study sites were comparable between islands and included low- to midelevation forest and second-growth habitats.

To represent continental South America, blood samples were obtained from 429 birds in northern coastal Venezuela and neighboring Trinidad, the closest landmasses to the Lesser Antilles. More than 3,000 samples were collected from the Greater Antillean islands of Jamaica, Puerto Rico, and Hispaniola. Parasite lineages from the Lesser Antilles were also compared to 240 parasite sequences obtained from North American birds sampled in southern Missouri, Alabama, southern Michigan, and Connecticut.

Screening and Sequencing

Birds were captured using mist nets, and 5–10 μL of blood were taken by wing or carotid venipuncture. We prepared smears using approximately 2–3 μL of blood. Slides were air dried, fixed in absolute methanol, and stained with Modified Giemsa Stain Solution (Sigma, St. Louis, MO). The remaining blood sample was stored in buffer until DNA extraction (see Seutin et al. 1991; Fallon et al. 2003*a* for details). Samples were collected and transported under the appropriate permits and licenses from local governments following protocols approved by the University of Pennsylvania and the University of Missouri–St. Louis.

We screened all extracted DNA samples for infection using a PCR assay based on a conserved RNA region of the 6-kb mtDNA genome of avian malaria (Fallon et al. 2003*a*). Blood smears were examined to confirm infections. We amplified a 363–base pair (bp) fragment of the parasite cytochrome *b* gene using the PCR with primers designed in our laboratory: 621F 5′ AAA AAT ACC CTT CTA TCC AAA TCT 3′ and 983R 5′ CAT CCA ATC CAT AAT AAA GCA T 3′ (see Richard et al. 2002; Fallon et al. 2003*b* for details and PCR conditions). We purified the amplified product by gel extraction. Sequencing was carried out in one direction on an automated sequencer (ABI Prism 377; Applied Biosystems, Foster City, CA) according to the manufacturer's protocol, yielding about 320 bp of sequence. The sequences were edited and aligned using Sequencher software (Gene Codes Corporation, Ann Arbor, MI) and are available through GenBank (accession numbers AY167239–AY167250, AY455656–AY455663, AY840997–AY841004).

Defining Parasite Lineages

We defined parasite lineages based on divergence in 320 sequenced nucleotides of the parasite's cytochrome *b* gene. Because most of these are not described taxa, lineage boundaries were defined arbitrarily, primarily on the basis of genetic variation. Lineages that we recognized as distinct differ from one another by between 1.2% and 14% sequence divergence (fig. 2), which exceeded the average level of intralineage variation by a factor of two or more. Within-lineage variation was minimal, averaging 0.6% (about 2 bp), and generally did not provide additional detail regarding host and island distributions of lineages. In one case, 3 bp (0.93%) differentiated two lineages in multiple individuals with distinct geographic distributions (lineages HJ and HD; table A2 in the appendix in the online edition of the *American Naturalist*). We considered these lineages evolutionarily independent. In two cases (HL and PI), a single base pair difference separated samples of multiple individuals that occurred in different hosts on different islands, and we considered these lineages evolutionarily independent, as well. Values of cytochrome *b* sequence divergence as low as 1.0% have been observed between named species of mammalian malaria parasites (Escalante et al. 1998), and lineages differing by as little as 0.5% exhibited complete linkage disequilibrium in one study (Bensch et al. 2004).

The generic identity of the parasite lineages (*Haemoproteus* [H] vs. *Plasmodium* [P]) was determined by the placement of cytochrome *b* sequences within a phylogenetic tree following Ricklefs and Fallon (2002) and Perkins and Schall (2002). We designated the parasite lineages arbitrarily by letter in alphabetic order. Three lineages recovered from doves (Cpa1, Cpa2, Zed) did not group unambiguously with either *Haemoproteus* or *Plasmodium* and thus have not been assigned to a genus at this point. Lineages HA-HI and PA-PF were described previously elsewhere (Fallon et al. 2003*b*, 2004).

Phylogenetic Analysis

We used Modeltest, version 3.06 (Posada and Crandall 1998), to determine the most appropriate evolutionary model for our data. The hierarchical likelihood ratio test selected the GTR + I + G model with the proportion of invariant sites = 0.4159 and gamma shape parameter = 0.7314. Empirical base frequencies were A = 0.3179, C = 0.1047, G = 0.1179, and T = 0.4595, and substitution rates were A-C 3.6403, A-G 3.9705, A-T 3.8970, C-G 0.9358, and C-T 21.4751, relative to G-T = 1.00. These settings were implemented in a maximum likelihood analysis to estimate the topology of the avian malaria parasite lineages recovered in the Lesser Antilles. The topology was also estimated using parsimony in PAUP, version 4.0b (Swofford 1998), and Bayesian inference in MrBayes 2.01 (Huelsenbeck and Ronquist 2001).

All methods produced similar topologies with only minor rearrangements involving lineages HB and PU1 and lineages within the basal clade of dove parasites. We present a 50% majority rule consensus tree based on the Bayesian analysis using the general time reversible model (Swofford et al. 1996) with partitioned codon positions and variable sites following a gamma distribution. We ran four Markov chain Monte Carlo chains for 1,000,000 generations, using random trees as starting points, sampling every hundredth generation, and discarding the first 1,000 trees as burn-in (fig. 2). An additional run with 2,000,000 generations, sampling every thousandth generation and discarding the first 500 trees, produced an identical topology and posterior clade probabilities. The tree was rooted using published sequences of *Leucocytozoon* spp. (see Perkins and Schall 2002).

Within each genus of parasite, we tested for an evolu-

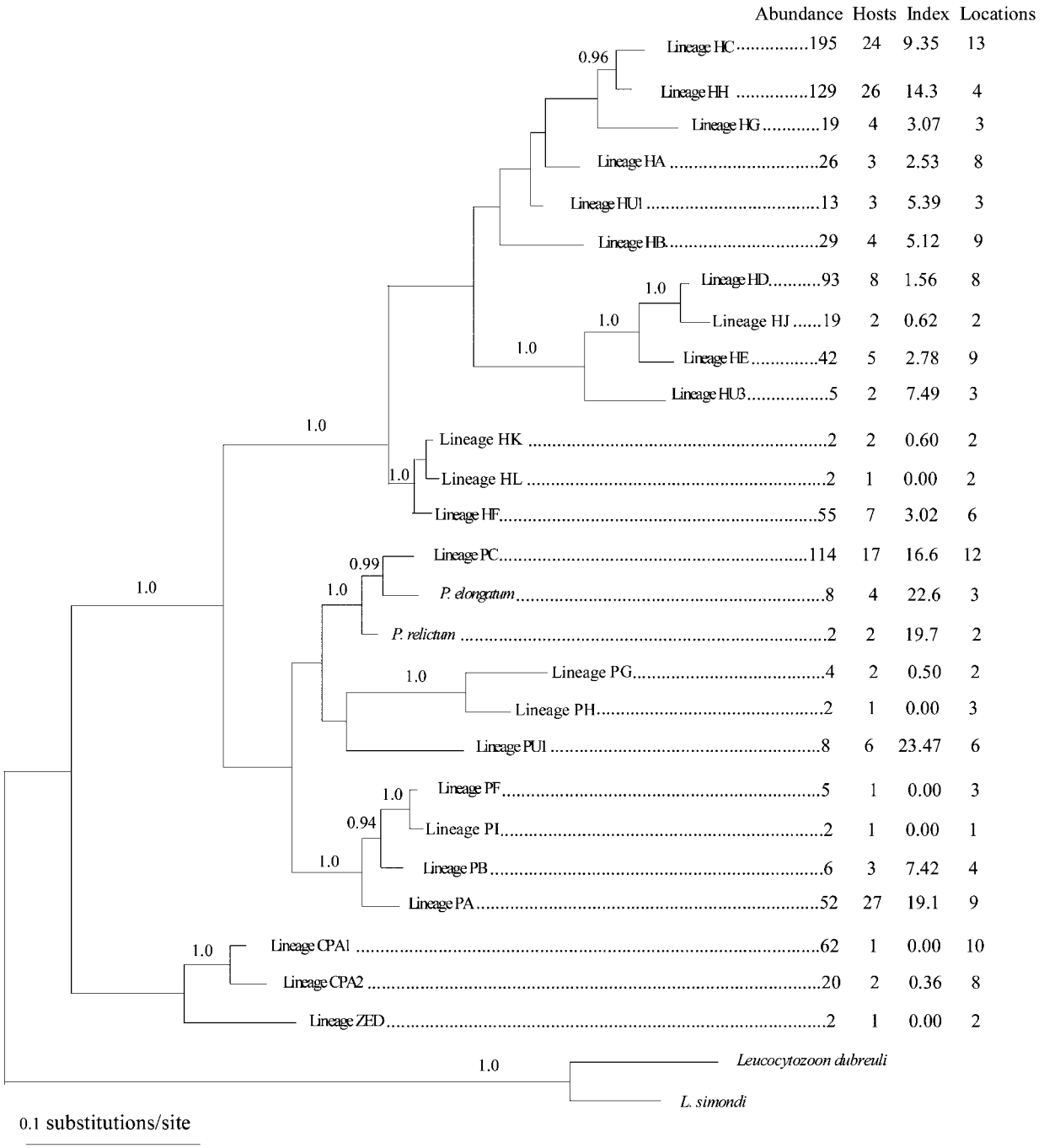

Figure 2: Phylogeny of Lesser Antillean parasite lineages based on 320 bp of the cytochrome *b* gene. Topology was estimated using Bayesian inference. Support values indicate posterior clade probabilities based on 9,000 Markov chain Monte Carlo sampled trees (see "Methods"). Numbers to the right indicate the abundance of the parasite lineage, the number of host species from which it was recovered, the lineage's estimated host breadth index, and the number of island and mainland locations from which it was sampled.

tionary relationship among the lineages' host breadth index values using Phylogenetic Independence, version 2.0 (Reeve and Abouheif 2003). The same analysis was used to evaluate the lineages' abundance.

Host Breadth Index

For each parasite lineage, we calculated a host breadth index based on the phylogenetic depth between all host species that the lineage infected. These values were weighted by the frequency with which the parasite lineage infected each host species. To estimate host depth, we used a measure of the relative genetic distance between host species. Because mitochondrial DNA in birds provides little resolution below the level of genus, we estimated the distance between the host species in our study following the phylogeny of Sibley and Ahlquist (1990) based on DNA hybridization (fig. 3 in the online edition of the *American Naturalist*). For the placement of species not included by Sibley and Ahlquist (1990), we used branching patterns from the phylogenetic studies of Lovette and Bermingham (2002) and Burns et al. (2002). Where relationships were unresolved or distances were not provided, we inserted a polytomy; species within the same genus were assigned distance values of 0.5 DNA hybridization units ($\Delta T_H 50$ [°C]). The resulting phylogeny was entered into Phenotypic Diversity Analysis Programs (PDAP, version 6.0; Garland et al. 1993) to produce a patristic distance (PDDST) matrix from the resulting host tree. The genetic distance between any two hosts (i, j) is d_{ij}. For each parasite lineage, we produced a separate probability matrix based on the frequency with which the lineage infected each host species. The contribution of two infected hosts (i, j) to the probability matrix is given by the product of the frequency of infection on each host, or $p_i p_j$. The host breadth index is the sum of the product of the comparable elements of the probability and distance matrices

$$\sum_{i=1}^{n} \sum_{j=1}^{n} d_{ij} p_i p_j.$$

This host breadth index is similar to a recently proposed host specificity index that incorporates taxonomic distinctness of infected host species (Poulin and Mouillot 2003). However, by estimating genetic distances between infected host species as well as accounting for the frequency of these infections (Rhode 1980), our index produces a more quantitative measure of specialization. Specifically, it estimates the average genetic distance between infected hosts in units of the melting point temperature. According to Sibley and Ahlquist's (1990) scale, family-level delineation among birds falls between 9° and 11°C $\Delta T_H 50$. Thus, low values for the index represent parasite lineages that primarily infected closely related hosts, while high values reflected parasite lineages that were found across deeply divergent host species.

Statistical Analysis

We used correlation analyses to test for relationships between the host-breadth index and the number of host species a parasite lineage infected, the parasite lineage's abundance as measured by the number of representative sequences for a particular lineage in our total sample, and its geographic range as measured by the number of geographic locations the lineage occupied (i.e., islands and continents).

The geographic heterogeneity of parasite distributions was assessed using *G*-tests followed by partitioned analyses (Sokal and Rohlf 1994). Because many parasite lineages are absent from at least one island, some cells in the contingency analyses contained zeros. To circumvent this problem, we added 0.1 to each cell. Adding 1 to each cell instead did not affect the overall outcome of the *G*-tests, although it decreased the sensitivity of the partitioned analyses for parasite samples with fewer than 10 occurrences.

We used multiple regression analyses (PROC GLM, SAS/STAT, version 8.0, SAS Institute, Cary, NC) to test for the effects of host phylogenetic age (young or old), distribution (widespread or restricted), geographic origin (north or south), and habitat on the prevalence of infections. We used Ricklefs and Bermingham's (2001, 2004) classification of host age, distribution, and geographic origin of host species. We followed Faaborg's (1985) classification of primary habitat (rainforest, dry forest, or widespread). To control for the strong species effect in the prevalence of infections and for uneven sampling, we weighted the number of infections for each host species by the square root of the host's sample size and arcsine transformed the prevalence data. Additionally, we tested for an effect of host phylogeny on each of the above traits (Phylogenetic Independence, version 2.0, McGill University, Montreal). Patterns of endemism were assessed using standard probabilities.

Results

We sampled 1,975 individual birds representing 53 species distributed among 10 islands in the Lesser Antilles, including Barbados (table A1). We detected 545 infections (28%) in 39 host species (74%) and successfully sequenced a region of the parasite mitochrondrial cytochrome *b* gene from 444 infected hosts (80% of infected individuals). Sequencing failures resulted from chromatograms that could

not be interpreted because of poor or unsuccessful amplification of the cytochrome *b* region due either to mixed infections (approximately 4%), mismatched priming sites, or weak template (Fallon et al. 2003*a*). In total, we identified 26 genetically distinct parasite lineages recovered from 29 host species representing 11 families of birds.

Distribution of Parasites among Hosts

Combined Prevalence. While the overall parasite prevalence averaged 28%, infections varied widely among host species, ranging from 0% to 100%. Nearly half of the species (47%) deviated significantly from the average prevalence; eight hosts exhibited higher than average rates of infection and 17 lower than average (fig. 4; table A3 in the appendix in the online edition of the *American Naturalist*). The differences in parasite prevalence were not attributable to host age in the Lesser Antilles, geographic distribution, or habitat use. Although parasite prevalence was affected by host phylogeny (C stat $= 0.484$, $P = .002$), all the other host traits considered were independent of host phylogeny (table 1).

Individual Parasite Lineages. Host breadth varied among genetically distinct avian malarial parasite lineages. Nearly 80% of the 26 Lesser Antillean parasite lineages were recovered from more than one host species across the entire geographic range of the study. Within the archipelago, the 26 parasite lineages were recovered from 29 host species. Regionally, we detected the same parasite lineages in an additional 37 host species. On average, parasite lineages infected 6.1 host species (range: 1–27).

To estimate the diversity of hosts from which particular parasite lineages were recovered, we calculated a phylogenetic index of host breadth for each parasite lineage that accounted for the depth of the relationships among hosts as well as a parasite lineage's prevalence on each species of host (see "Methods"). The index ranged from 0 for parasite lineages that were recovered from only one host species to 23.5 for lineage PU1 (six different host species from diverse families, including Tyrannidae, Vireonidae, and Fringillidae). The mean host phylogenetic index for the 26 parasite lineages was 6.36; the median was 2.9.

Despite multiple hosts, many parasite lineages primarily infected only a few species of the same host family or subfamily. For example, lineages HD, HJ, and HE averaged only 1.66 for the host breadth index because individually these parasite lineages were nearly exclusive to just one or two fringillid host species (*Coereba flaveola* and *Loxigilla noctis*). Parasite lineages HA, HB, HU1, and PB were recovered primarily from vireos (Vireonidae), while lineages Cpa1 and Cpa2 were exclusive to the ground doves *Columbina passerina* and *Columbina talpacoti*. We recovered nine other lineages almost exclusively from either thrasher (Mimidae) or thrush (Turdidae) hosts endemic to the West Indian region.

The remaining parasite lineages were more evenly distributed across multiple host families, and all had host breadth indexes exceeding 9.0, which corresponds to family-level delineation for the host species (Sibley and Ahlquist 1990; see fig. 5). We refer to these lineages as generalist parasites. Lineage HC, with a host index of 9.35, largely infected fringillids from the subfamily Emberizinae (87%), though across its entire range, it was recovered from a total of 24 host species from eight families. Lineage HH was found in five host families on Grenada. Across the rest of its geographic range lineage, HH infected an additional 15 species from two other families. Lineage PA was found in 51 host individuals belonging to 27 species. Lineage PC, the third most abundant lineage in the Lesser Antilles, mostly infected emberizids, but 40% of infections from this lineage came from an additional 12 species in three other host families. The remaining three parasite lineages were recovered in small numbers but from diverse hosts. Lineage PU1 infected eight individuals from four different families. The other two parasite lineages match *Plasmodium elongatum* and *Plasmodium relictum* (GenBank accession numbers AF254975, AF069611) and are labeled as such in figure 2. *Plasmodium elongatum* was recovered from three host families, and *P. relictum* was recovered from only two individuals, each from a different family.

Because host family distribution increases with the number of host species, the index of host breadth was positively correlated ($r = 0.51$, df $= 24$, $P < .05$; fig. 5). However, the index was not correlated with the overall abundance of a lineage ($r = 0.21$, df $= 24$, $P > .05$) or its geographic distribution ($r = 0.19$, df $= 24$, $P > .05$). Both abundance and specialization (as measured by host breadth) were phylogenetically independent (Reeve and Abouheif 2003) for both *Haemoproteus* and *Plasmodium* parasites (table 2). When the two genera were considered simultaneously, abundance remained phylogenetically independent, but host breadth did not, owing to the greater specialization of *Haemoproteus* lineages (average host breadth index $= 4.29$) compared with *Plasmodium* lineages (average $= 10.94$).

Geographic Distributions

Combined Prevalence. Island-wide prevalence of infection varied across the archipelago (fig. 6; $G = 92.4$, df $= 9$, $P < .001$). Montserrat exhibited significantly elevated prevalence compared with other islands. This heterogeneity was due in large part to elevated infections in three mimid species (*Margarops fuscatus*, *Margarops fuscus*, and *Cinclo-*

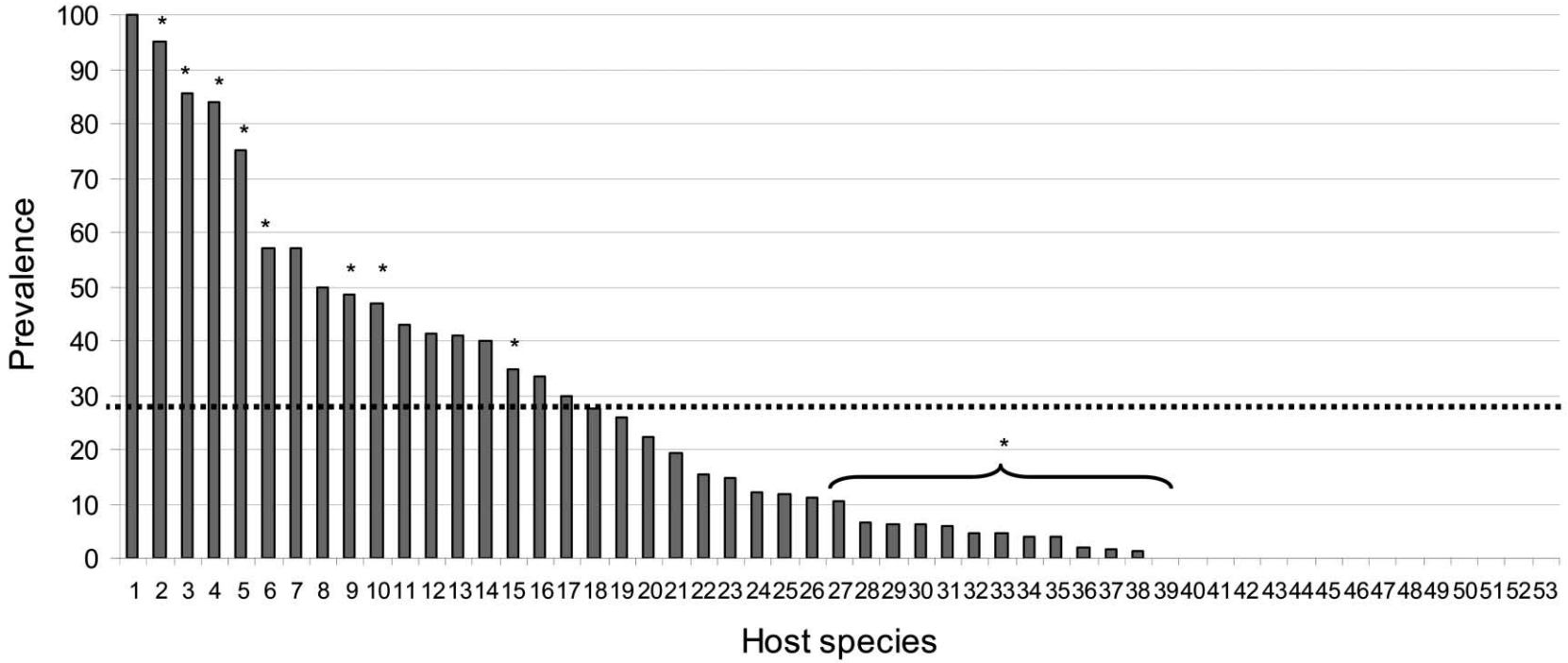

Figure 4: Overall prevalence of infection by host species. Asterisks denote values that differed significantly from the average of 28%. Species names, total sample size, and corresponding *G* values can be found in table A3 in the online edition of the *American Naturalist*.

Table 1: Phylogenetic independence of host traits

Variables	*C* statistic	*P* value
Parasite prevalence	.483	.002
Phylogenetic age	.200	.055
Geographic distribution	.066	.261
Northern/southern origin	.079	.242
Habitat	−.172	.064

certhia ruficauda; $G = 13.7$, df = 1, $P < .001$) as well as common ground doves (*C. passerina*; $G = 13.7$, df = 1, $P < .001$). When these species were removed from the analysis, parasite prevalence decreased to an average of 24% on each of the islands, although prevalence remained heterogeneous across islands: higher on Guadeloupe and St. Lucia and relatively lower on Grenada (partitioned *G*-test: $G = 13.5$, 6.6, 15.1, respectively, df = 1, $P < .05$). The central core islands of Guadeloupe, Dominica, Martinique, and St. Lucia had statistically indistinguishable prevalences of malaria infection ($G = 5.4$, df = 3, $P > .05$) averaging 31%, while the lower-lying islands (Antigua, Barbuda, and Barbados) shared similar but lower frequencies of infection at about 21% ($G = 1.4$, df = 2, $P > .05$). St. Vincent and Grenada also shared similar lower frequencies of infection ($G = 2.5$, df = 4, $P > .05$).

Individual Parasite Lineages. Despite comparable prevalence of infection within the core islands, among the low islands, and on Grenada and St. Vincent, the geographic distribution of individual parasite lineages varied significantly within each of these island groups (table A2). Because some parasite lineages demonstrate significant host specificity, variation in the distribution of lineages is, in part, explained by variation in the sampling of particular host species on each island. Therefore, we analyzed geographic heterogeneity of parasite lineages only for cases having sufficient sampling of appropriate host species. For example, the core islands share a similar set of parasite lineages, with slight variations accounted for by uneven sampling of hosts. Lineage Cpa1, which was recovered exclusively from the common ground dove *C. passerina*, was missing from the St. Lucia sample, where this host was not caught. Similarly, parasite lineages HF and HG were recovered mostly from mimid species, of which we caught no parasitized individuals on Martinique. We therefore removed these three lineages from the analysis. The distribution of the remaining 18 parasite lineages did not vary significantly between the four central islands ($G = 62.36$, df = 51, $P > .05$). Neighboring Montserrat shared most of the same lineages with the core islands but was noticeably missing the second most common lineage, PC ($G = 91.1$, df = 68, $P < .05$). This difference cannot be accounted for by the sampling of hosts because lineage PC infects a wide variety of bird species that are well represented on Montserrat. However, the remaining 17 lineages did not differ in their distributions across these five islands ($G = 72.2$, df = 64, $P > .05$).

The lower-lying islands exhibited some similarities in parasite communities. Antigua, Barbuda, and Barbados all had significantly fewer parasite lineages than did the other islands. In fact, only one parasite lineage, HC, was recovered from Barbados. In addition, Antigua and Barbuda both harbored lineages HA and HE, though these parasites were rare within the archipelago and were missing from six of the remaining eight islands despite the presence of appropriate hosts. The peripheral islands also were distinguished by significantly greater numbers of *Haemoproteus* infections. Approximately 53% of infections on the four central islands were *Plasmodium*, while 90%–100% of infections on the six peripheral islands were *Haemoproteus*.

Beyond this pattern in the distribution of the parasite genera, the distribution of individual *Haemoproteus* parasite lineages varied across the peripheral islands. For example, lineage HD, common on both Barbuda and Montserrat, was conspicuously missing from Antigua despite the presence of appropriate host species ($G = 9.19$, df = 1, $P < .05$). Also, while we recovered only one parasite lineage from Barbados, a similar sample of hosts on neighboring St. Vincent yielded seven lineages. Nonetheless, St. Vincent lacked a number of common parasite lineages while harboring two lineages not found elsewhere within the Lesser Antilles but present in North America and, in one case, in South America as well. Last, Grenada stands out in its distribution of parasite lineages in that three of its 10 lineages were not recovered on any other Lesser Antillean island. Even more striking, these three parasite lineages from this southernmost Lesser Antillean island occurred in the Greater Antilles, and one of these occurs in North America (table A2). Four additional lineages that were common on the other Lesser Antillean islands, as well as occurring on the mainland of Venezuela, were not detected on Grenada despite the presence of appropriate hosts and extensive sampling.

Regional Distributions. In a regional survey of parasite lineages, the 26 lineages found in the Lesser Antilles were detected in an additional 465 individuals from Venezuela (South America), the Greater Antilles, and North America. Regional distributions of the parasite lineages varied from being localized to one island to being broadly distributed across the entire archipelago and elsewhere. The most abundant parasite lineage (HC) was the only lineage to be found on every island in the Lesser Antilles and accounted for 32% of all infections in the archipelago. Lineage HC was also recovered in the Greater Antilles and in small numbers in both North and South America. Five other

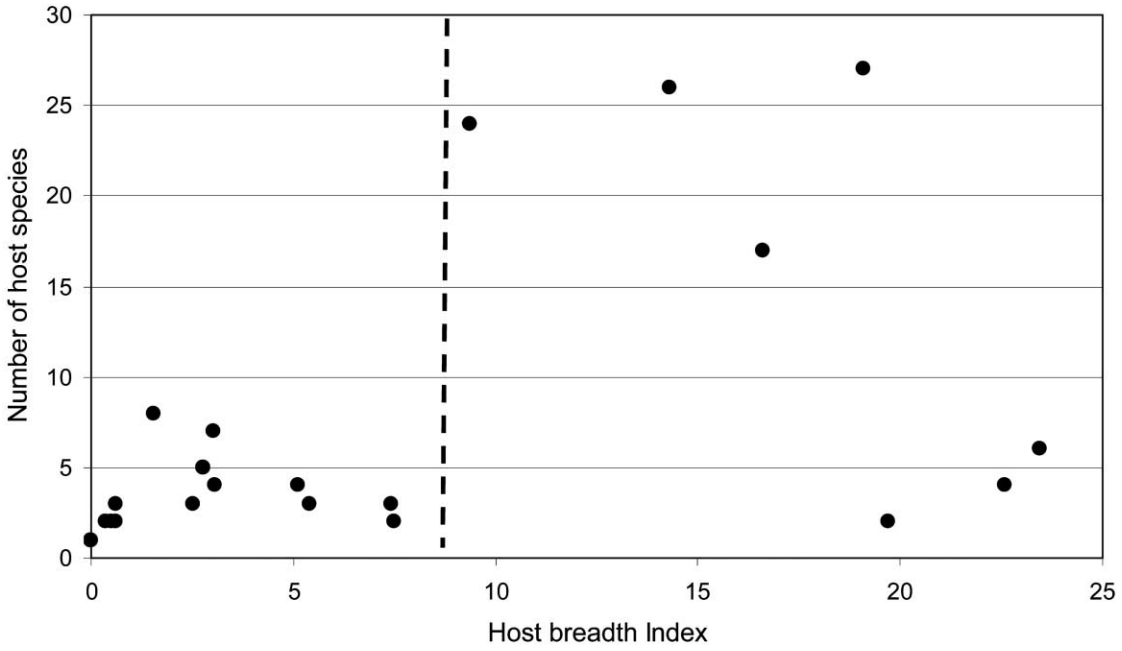

Figure 5: Correlation between phylogenetic host breadth index and the number of hosts infected by a particular parasite lineage ($r = 0.51$, df = 24, $P < .05$). Dashed line corresponds to family-level delineation for host species based on DNA hybridization analyses by Sibley and Ahlquist (1990; see also fig. 3 in the online edition of the *American Naturalist*). Values with a host breadth index greater than 9 are considered generalist parasites.

lineages were found across all four geographic regions (table 3). All together, 20 Lesser Antillean parasite lineages were found in at least one region outside of the archipelago: 15 in the Greater Antilles, 14 in Venezuela, and 11 in North America.

Endemism. Despite the wide distribution of many of the Lesser Antillean parasite lineages, six were recovered only from within the archipelago and likely represent endemic parasites. On the basis of the distribution of the Lesser Antillean parasite lineages in North America, the Greater Antilles, and South America, the probability under random distribution of any given parasite lineage not occurring outside of the Lesser Antilles is 0.108. Accordingly, more parasite lineages ($6/26 = 0.230$) are restricted to this region than predicted by chance (table 3).

Five of these geographically restricted parasite lineages were recovered almost exclusively from endemic hosts: three mimids, *M. fuscatus*, *M. fuscus*, and *C. ruficauda*, and the forest thrush *Cichlherminia lherminieri*. The sixth endemic parasite lineage was recovered from a nonendemic host, *Zenaida aurita*, which was not well sampled in other geographic regions.

Discussion

Host Associations

Nearly 75% of the Lesser Antillean parasite lineages demonstrated some level of host specificity, being primarily recovered from a small group of closely related hosts. Nonetheless, seven of the 26 parasite lineages had a host breadth index >9, indicating infection of hosts separated on average at the family level. While the three most abundant parasite lineages were considered generalist parasites by this measure, the remaining four generalists were present in low numbers, and some specialist lineages were relatively abundant (i.e., lineages HD and Cpa1). Therefore, abundance was not correlated with the host breadth index, suggesting that generalization does not necessarily confer an advantage in terms of a parasite's ability to achieve high occurrence. Furthermore, the phylogenetic age of the hosts, their habitat, geographic distribution, and origin also did not explain variation in parasite prevalence.

Although specialization at the family or subfamily level is common, strict host specialization at the species level is relatively rare. Only six lineages were recovered exclusively from one host species and except for the common ground dove lineage (Cpa1), which was recovered in large numbers, the five remaining specialist lineages were found in

Table 2: Phylogenetic independence for abundance and host breadth index for parasite lineages within each genus

Variables	*C* statistic	*P* value
Haemoproteus:		
Abundance	.255	.110
Host breadth index	.204	.138
Plasmodium:		
Abundance	−.042	.470
Host breadth index	.337	.102
All lineages combined:		
Abundance	.0744	.258
Host breadth index	.299	.019

only two to five individuals of three host species: *Turdus plumbeus* (two lineages), *Cichlherminia lherminieri* (two), and *Zenaida aurita* (one). The second and third of these hosts are relatively uncommon and were poorly sampled in this study ($N = 14$ and 10, respectively). However, 62 individuals of *T. plumbeus* sampled across three islands (Dominica, Puerto Rico, and Hispaniola) yielded only 12 infections, of which we sequenced 10, nine of which belonged to a host specialist. Thus, strict specialization on one host species is not generally associated with high abundance.

Within each parasite genus, the degree of specialization is phylogenetically independent, indicating the lability of host breadth over parasite evolutionary history. This pattern stands in contrast to the idea that parasites evolve toward increased specialization (Mayr 1942; Futuyma and Moreno 1988). However, *Haemoproteus* parasites exhibit greater specialization than do *Plasmodium* parasites (see "Results"). Because *Plasmodium* parasites are paraphyletic with respect to *Haemoproteus*, this is consistent with the notion that generalists give rise to specialists. The most common pattern of infection observed in our data was an intermediate level of specialization, primarily within a host family or subfamily, supporting previous reports that parasite host sharing (or switching) occurs most often between closely related hosts (Bensch et al. 2000; Ricklefs and Fallon 2002; Beadell et al. 2004).

Geographic Patterns

Although the distributions of avian malaria parasites are constrained by their hosts, the independence of parasite and host distribution observed in this study indicates that other factors influence distributions. The four core islands of Guadeloupe, Dominica, Martinique, and St. Lucia have similar prevalence and lineages of parasites. However, the remaining peripheral islands vary in both respects. *Barbados*. Patterns of infection on the peripheral islands provide insights into the dynamics that shape the geographic distributions of parasite lineages. For example, that only one parasite lineage (HC) was recovered from Barbados stands in stark contrast to the diversity of lineages encountered elsewhere in the archipelago. Barbados is geographically isolated (fig. 1) and relatively young, having been subaerial for approximately 700,000 years (Mesolella 1967; Mesolella et al. 1970; Bender et al. 1979). Mitochondrial DNA evidence suggests that most of the bird populations on this island colonized more recently, likely having arrived from neighboring St. Lucia and St. Vincent within the last 200,000 years (Lovette et al. 1999*b*). Although the timing of founding events varied, six of eight bird species examined showed significant genetic differentiation from their source populations, indicating that successful colonization of Barbados by host individuals is rare and that host populations are too small to retain genetic variation.

The absence from Barbados of all but one of the parasite lineages that occur on the main Lesser Antillean chain of islands to the west would indicate either that the host species on Barbados have lost parasite lineages that they originally brought with them or that founding hosts did not carry other parasite lineages with them when they colonized. "Missing the boat" events are more likely in isolated areas when the distribution of infections on their hosts is patchy (Paterson et al. 1999). Also, the absence of *Plasmodium* lineages might reflect a lack of appropriate vectors. However, mosquitoes capable of transmitting avian *Plasmodium* (*Culex quinquefasciatus* and other *Culex* spp.) have been reported from Barbados (Belkin and Heinemann 1976). Because eight of the host species on Barbados harbor a combined total of nine parasite lineages on St. Lucia (including lineage HC), it is unlikely that colonizing hosts did not carry any of the remaining eight lineages with them. Accordingly, the occurrence of a single parasite lineage on Barbados suggests that most of the original parasite diversity on Barbados likely has disappeared.

Three recent colonists to Barbados (*Tiaris bicolor*, *Columbina passerina*, and *Quiscalus lugubris*; Lovette et al. 1999*b*; E. Bermingham and R. E. Ricklefs, unpublished data) are typically infected elsewhere by parasites other than the HC lineage found on Barbados. Indeed, of 85 sequenced infections of *C. passerina* and 53 of *Q. lugubris* from other locations, none was infected with lineage HC. The frequency with which *T. bicolor* is infected with lineage HC is also relatively low (16%) across its entire range. Thus, the acquisition of HC by *C. passerina* and *Q. lugubris* on Barbados appears to represent an expansion in host range (i.e., host switching) for the parasite. Moreover, the absence of parasite lineages other than HC on Barbados

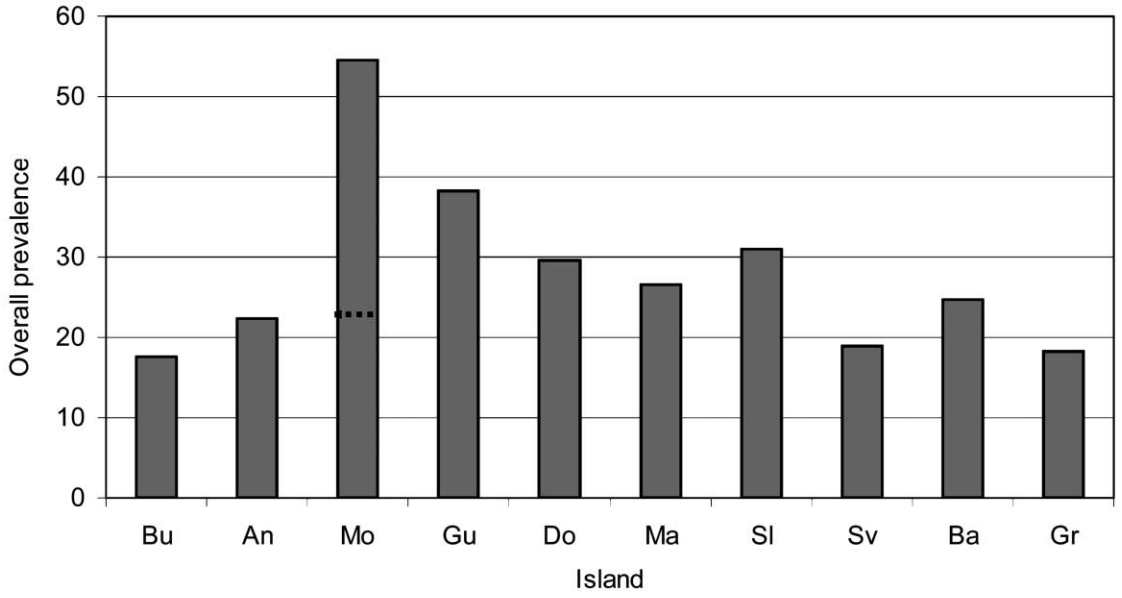

Figure 6: Overall prevalence of infection by island. Dashed line on Montserrat represents the adjusted measure of prevalence in the absence of elevated infections from four host species (see "Results"). Islands are arranged from north to south. *Bu* = Barbuda, *An* = Antigua, *Mo* = Montserrat, *Gu* = Guadeloupe, *Do* = Dominica, *Ma* = Martinique, *Sl* = St. Lucia, *Sv* = St. Vincent, *Ba* = Barbados, *Gr* = Grenada.

suggests that host switching might be facilitated by the absence of competition with other parasite lineages.

Grenada and St. Vincent. Extinction also is likely to be a feature of the malaria parasite fauna on the southern islands of Grenada and St. Vincent, which are missing several lineages that are common throughout the rest of the Lesser Antilles and South America. Alternatively, hosts carrying these parasites might have bypassed these islands in dispersing through the archipelago. This scenario is unlikely because avian colonization of the Lesser Antilles has occurred overwhelmingly in stepping-stone fashion (Ricklefs and Bermingham 1999, 2002). That St. Vincent and Grenada do not themselves share many parasite lineages is also striking. During glacial sea level lows (approximately 18,000 years ago), Grenada extended to within a few kilometers of St. Vincent (Pregill and Olson 1981; Fairbanks 1989), which would have facilitated the dispersal of birds (and parasites) between the two islands. If these island communities were nearly homogeneous at that time, both hosts and parasites have since diverged considerably. Because parasite lineage turnover might occur on timescales as short as a decade (Fallon et al. 2004), the divergence of parasite communities between these islands is not unexpected. However, the high degree of sharing of parasite lineages between other islands in the archipelago would suggest that current dispersal between St. Vincent and Grenada, as well as between these islands and the rest of the archipelago, is limited.

Antigua and Barbuda. The northern peripheral islands of Antigua and Barbuda also exhibit unique assemblages of parasite lineages. Antigua and Barbuda shared a land connection during glacial sea level lows (Pregill and Olson 1981; Fairbanks 1989). Except for the absence of lineage HD from Antigua, their parasite communities are similar. Assuming the host and parasite communities on these two islands were once homogeneous, and considering that lineage HD is present to the south of Antigua as well, the absence of this parasite lineage from Antigua is most easily explained by extinction without subsequent recolonization.

The abundance of lineage HE on these northern islands is striking considering its near absence from much of the rest of the archipelago. Furthermore, lineage HE is strongly associated with a common and widespread endemic host, *Loxigilla noctis*, which was well sampled elsewhere. Lineage HE was found in the Dominican Republic and the mainland of Venezuela. However, large and significant gaps in its distribution across the Lesser Antillean islands, despite being associated with a common host, are also consistent with historical extinction events and limited recolonization.

Table 3: Regional distribution of avian malarial parasite lineages

North America	Greater Antilles	Lesser Antilles	South America	*N*	No. lineages
		34		34	6*
		4	4	8	1
1		1		2	1
	23	5		28	4
17		51	6	74	3
17	95	17		129	1
	110	76	31	217	4
55	85	256	28	424	6
90	313	444	69	916	26

* $P = .05$, significantly different from random distribution.

Endemism

Several parasite lineages are restricted to the Lesser Antillean archipelago in association with old endemic host species. Four species of thrashers in the family Mimidae (*Margarops fuscatus*, *Margarops fuscus*, *Cinclocerthia ruficauda*, and *Ramphocinclus brachyurus*) represent a 4-million-year-old endemic radiation within the Lesser Antillean archipelago (Hunt et al. 2001). However, the three endemic parasite lineages recovered from three of these hosts (lineages HU3, HG, and PG in fig. 2) are derived from unrelated branches of the parasite phylogeny and thus do not represent a corresponding radiation. One lineage in particular (HG) differs considerably (3.6%) from its next nearest neighbor in a regional phylogenetic analysis of parasite lineages from North America, the Greater and Lesser Antilles, and Venezuela, indicating a long, isolated evolutionary history (results not shown). If this parasite had been associated with its endemic hosts for the duration of the hosts' history in the region, the genetic differentiation in the parasite lineages relative to their hosts (approximately 8%) would support the contention that parasite cytochrome *b* sequences evolve more slowly than those of their hosts (Ricklefs and Fallon 2002). However, if parasite sequence divergence were more rapid, then the endemic parasites would be younger than their hosts and acquired after the mimids entered the archipelago, or continued gene flow between parasite populations on different hosts would delay their divergence. The two endemic parasite lineages recovered from the forest thrush *C. lherminieri* are each only 0.3% (1 bp) differentiated from parasite lineages (HK and PF, respectively) recovered from other hosts in the family Turdidae. Lineages HK and PF were rare ($N = 2$ and 5) and occurred on other islands both within and outside the Lesser Antillean archipelago. Whether the lineages recovered from the forest thrush represent distinct parasite species would be difficult to determine even if they were sympatric with their close relations. However, additional examples of host and/or geographic range disjunctions suggest that the distributional patterns of parasite lineages may reveal evolutionary differentiation reflected by little or no mitochondrial variation.

Disjunct Distributions

The association of avian malaria parasite lineages with a particular host species or group of species is, for the most part, consistent across the geographic range of the parasite. However, a few lineages exhibit disjunct host and geographic distributions. The most striking example is lineage HH, which was recovered from North America, the Greater Antilles, and Grenada but was not found on the rest of the intervening Lesser Antilles. This distribution might be a relict of a once widespread parasite lineage that has since disappeared from the other islands, perhaps by exclusion due to the presence of other parasite lineages. Parasite lineage HC, for example, is abundant on all the Lesser Antillean islands where lineage HH is missing but is absent from a large sample in the Dominican Republic where lineage HH is present. Spatial and temporal patterns of avian malarial infections between populations of parasites indicate that lineages compete with each other for infections and may on occasion displace each other (Bensch and Akesson 2003; Fallon et al. 2003*b*, 2004). Therefore, a scenario in which disjunct geographic distributions result from competition between lineages is consistent with the community dynamics of avian malaria parasites. However, because lineages HH and HC are found together in North America, Puerto Rico, and Grenada, factors other than competition might have excluded lineage HH from the Lesser Antilles.

It is also striking that each of these parasite lineages infects not only different species but also different families of hosts in North America, the Greater Antilles, and Grenada. For example, in North America, lineages HC and HH were obtained almost entirely from fringillids and a few vireos. In the Greater Antilles, we found one of these parasite lineages on a tyrannid, and in Grenada, these parasites were found on four additional families of birds. The only overlap between HC and HH concerned one host species (*Coereba flaveola*) and two families (a vireo and a tyrannid were infected by both in Grenada).

The North American bird sample is composed largely of Neotropical migrants that could pick up infections while overwintering in the Greater Antilles, though the host disjunctions between Grenada and the Greater Antilles suggest sufficient time and isolation for differentiation of the parasite lineages in terms of host preference. Grenada is distinctive in having been colonized by a number of South American bird species that do not extend further north

and in missing a few of the core Lesser Antillean birds. Therefore, there are a number of differences between the host assemblages of Grenada and the Greater Antilles, as well as the remainder of the Lesser Antilles. Nevertheless, Grenada also harbors many of the same hosts species or families, and so the switch in host use at the family level between Grenada and the Greater Antilles is notable. The parasite assemblage of the Greater Antilles includes all of the lineages found in Grenada. However, Grenada lacks a number of lineages found in the north. The resulting differences between the parasite assemblages may be sufficient to affect the host distributions of the parasite lineages in these regions.

These host and geographic disjunctions occur in the absence of genetic differentiation for a portion of the parasites' mitochondrial cytochrome *b* gene. Clearly, molecular evolution as measured in this study proceeds more slowly than the dynamics of host and geographic distribution within the parasite lineages, whether because of intrinsically slow evolution or because continued gene flow between parasite populations on different hosts slows evolutionary divergence.

Geographic Origins

Last, despite the relative frequency of extinction relative to colonization in avian malaria, regional distributions of parasite lineages in this study highlight the ability of parasites to disperse great distances and may provide evidence of the geographic origins of some parasites. For example, the dissimilarity in the parasite communities of Grenada and South America, combined with connections to the Greater Antillean islands, suggests that many of the Lesser Antillean parasites originated from the north and traveled southward. Although Grenada shares a number of host species with South America, this southern island is missing seven of the Lesser Antillean parasite lineages that were recovered in Venezuela and harbors four parasite lineages not found on the southern mainland. Also, only one of the Lesser Antillean parasite lineages has an exclusive connection to South America, whereas several have exclusive connections to the Greater Antilles or North America (table 3). This is consistent with the history of a number of host species in the region including the most common and widespread *Loxigilla noctis* and *C. flaveola*, which appear to have spread to the Lesser Antilles from the north (Seutin et al. 1996; R. E. Ricklefs and E. Bermingham, unpublished data).

Conclusions

The Lesser Antilles harbor a diverse community of avian malarial parasite lineages that infect 75% of the resident species sampled in this study. Infection patterns do not appear to be explained by various ecological traits of their host species; however, parasite prevalence is related to host phylogeny, suggesting that infection by malaria parasites might depend on evolutionarily conservative aspects of the host, such as immune response. Future studies should also consider the relationship and distribution of malaria parasites with their invertebrate vectors—an area of study that is generally lacking.

Parasite lineages were recognized in this study by nucleotide substitutions in a portion of the mitochondrial cytochrome *b* gene. This level of genetic resolution is associated with significant variation in the distribution of parasites with respect to host species and geography, reflecting processes of host switching, dispersal, and extinction of populations on hosts or islands that occur on a longer timescale than cytochrome *b* differentiation. Endemic lineages found primarily in endemic hosts provide evidence for a long evolutionary history in the region. However, much of the change in host and geographic distribution has occurred too recently to leave an imprint in the cytochrome *b* gene. Many parasite lineages were absent from islands despite the availability of appropriate hosts, suggesting frequent local extinction events relative to dispersal, which appears to be limited. For example, both the northern peripheral islands (Antigua and Barbuda) and the southern ones (Barbados, St. Vincent, and Grenada) demonstrate unique features to their parasite communities indicative of isolation and differentiation. Disjunctions in host use and geography reveal differentiation not detected by lineage designations.

Consequently, host and geographic differentiation of parasite lineages, including extinction, host switching, and recolonization, can occur more rapidly than mitochondrial divergence. At the same time, many lineages have broad geographic ranges reaching both North and South American continents, indicating an ability to disperse great distances. Despite this ecological dynamism, however, a certain level of evolutionary stability has resulted in the nonrandom distribution of avian malarial parasites with respect to their hosts and geography.

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