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ISLAND AND TAXON EFFECTS IN PARASITISM REVISITED: AVIAN MALARIA IN THE LESSER ANTILLES

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Abstract.—We identify and describe the distribution of 12 genetically distinct malaria parasite lineages over islands and hosts in four common passerine birds in the Lesser Antilles. Combined parasite prevalence demonstrates strong host effects, little or no island effect, and a significant host-times-island interaction, indicating independent outcomes of host-parasite infections among island populations of the same host species. Host- and/or island-specific parasite lineages do not explain these host-parasite associations; rather, individual lineages themselves demonstrate the same type of independent interactions. Unlike overall prevalence, individual parasite lineages show considerable geographic structure (i.e., island effects) as well as species effects indicating that parasite lineages are constrained in their ability to move between hosts and locations. Together, our results suggest an upper limit to the number of host individuals that malaria parasites, as a community, can infect. Within this limit, however, the relative frequency of the different lineages varies reflecting fine scale interactions between host and parasite populations. Patterns of host-parasite associations within this system suggest both historical co-evolution and ecologically dynamic and independent host-parasite interactions.

Key words.—Avian malaria, coevolution, *Haemoproteus*, host-parasite interactions, Lesser Antilles, passerines, *Plasmodium*.

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The distribution of parasites geographically and among potential host species presumably reflects the population and evolutionary dynamics of host-parasite interactions (Anderson and May 1978; Poulin 1993; Thompson 1994). However, the factors shaping these relationships are sometimes ambiguous. For example, specialized parasites with localized distributions, few hosts, or both, might exploit their hosts more successfully than widespread, generalized parasites. Alternatively, their narrower endemism might result from lower efficiency as parasites. According to the idea that trade-offs in life histories influence competitive ability (Petraitis et al. 1989; Tessier et al. 2000), more generalized parasites presumably encounter a wider range of physical conditions, vectors, and host defense mechanisms, adaptation to which might compromise their efficiency as parasites. Furthermore, the success of a parasite on a single species of host might vary across the host's range due to geographically variable selection between populations (Thompson 1994, 1999).

In the case of malarial blood parasites, parasite prevalence, which is the proportion of individuals parasitized in a population of hosts, is a common measure used in describing parasite distributions. However, few studies of blood parasites have addressed the issue of prevalence across both host species and geographically separated host populations. Furthermore, because most studies of parasites in nonhuman populations use a morphologically based taxonomy, they cannot detect independently evolving strains or lineages of parasites within which specialization might occur. As a result, the co-evolutionary dynamics of this host-parasite system are largely unexplored in nonhuman populations. Only with the application of PCR and sequencing has it become possible to characterize lineages of malaria parasites genetically and begin to analyze geographic and host distributions in malaria species (Bensch et al. 2000; Perkins 2000, 2001; Ricklefs and Fallon 2002; Waldenström et al. 2002). In this study, we analyze variation between islands and hosts in the prevalence of several lineages of avian malaria (*Haemoproteus* and *Plasmodium*) in the Lesser Antilles, and demonstrate strong differentiation in the host-parasite relationship among islands and host populations.

Avian malaria represents a complex, spatially heterogeneous host-parasite system having ecological and evolutionary impact on host populations (van Riper et al. 1986; Atkinson and van Riper 1991). At one extreme, malaria parasites have been implicated in the extinction or decline of several species of birds in Hawaii (van Riper et al. 1986; Atkinson et al. 1995, 2000; Massey et al. 1996). Additional studies of the interactions between blood parasites, mate choice, reproductive success, and immune response indicate that malaria may be a significant selective factor in bird populations (Korpimaki et al. 1995; Dale et al. 1996; Dufva 1996; Allander 1997; Siikamaki et al. 1997; Nordling et al. 1998; Ots and Horak 1998; Wiehn et al. 1999). The ecological and evolutionary consequences of malarial parasites and their heterogeneous distribution among hosts and across geographic locations make for a compelling system in which to address questions regarding the complex host-parasite association. A reasonable first step in this direction is to determine whether parasites are randomly distributed or exhibit patterns that might be caused by ecological variation in their environments, co-evolution with their hosts, or accidents of history.

Archipelagoes provide excellent study systems for teasing apart ecological and evolutionary effects by allowing for replicated evolutionary events between ecologically similar, yet discrete locations. This is particularly true when the same potential host species occur on all the islands. In analyses of variance of parasite prevalence conducted in such a system, host species' effects represent unique genetic and ecological attributes of hosts or host-specific parasites. Depending on the stability of these effects in space and time, they may

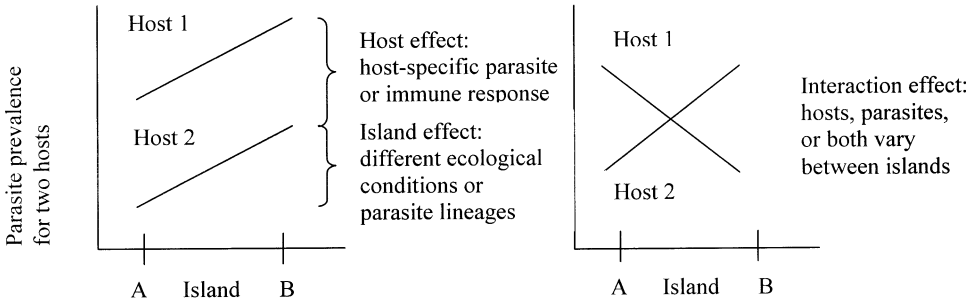


FIG. 1. Schematic representation of host, island, and interaction effects. See text for details.

either be repeated across islands, or vary between locations. Significant island effects on host prevalence would imply ecological differences in the environments of islands that affect the transmission of parasites regardless of parasite lineage or host identity. A statistically significant interaction between host and island effects would reveal independent outcomes of the host-parasite interaction within individual island populations of each host (Fig. 1). This independence could reflect unique adaptations of either the host or parasite populations on an island that influence parasite prevalence, or it might result from variation in the parasite lineages that infect different hosts on different islands.

In a recent study, Apanius et al. (2000) analyzed the prevalence of malaria blood parasites in populations of six species of birds distributed across three islands in the Lesser Antilles. This study revealed a significant host-times-island interaction in parasite prevalence, which provided support for the evolutionary independence of host-parasite associations between the different island populations. Because parasite prevalence demonstrated a species effect, but no island effect, the heterogeneity of parasite infections led the authors to suggest that genetic differences in host resistance or parasite virulence, rather than special ecological factors involving local host-parasite relationships, were responsible for the significant interaction. However, mitochondrial DNA sequences revealed no detectable genetic differences between island populations of any of the six host species, indicating either mitochondrial variation is not the appropriate measure of differentiation in this host system, or parasite genetic differences are responsible for the variation in parasite infection (Apanius et al. 2000).

Here, we revisit the study system of Apanius et al. (2000) using molecular techniques to detect parasite infections and identify individual parasite lineages within the same island populations of birds, and in some cases, the same individual, included in the original study. We begin by determining the stability of the host and island statistical patterns across a wider geographic range within the Lesser Antilles. We then test the hypothesis that distinct parasite lineages are responsible for host-times-island interactions. In doing so, we describe host and island affinities of avian malarial parasite lineages for the first time and begin to unravel the complex co-evolutionary dynamics of this system. We confirm the previous finding of independent host–parasite associations and present additional data on island and taxon effects as they relate to specific parasite lineages.

MATERIALS AND METHODS

Natural History of the Host and Parasite

Both *Haemoproteus* and avian *Plasmodium* parasites are globally distributed (Atkinson and van Riper 1991), including the West Indies (Apanius et al. 2000; Bennett et al. 1980). They are intracellular parasites in the suborder Haemosporina within the protozoan phylum Apicomplexa. Members of the phylum receive their name from an apical complex of organelles that aids in cell penetration. They also share developmental characteristics related to their life cycle of alternating phases of sexual and asexual reproduction that require both a vertebrate host and an arthropod vector (Garnham 1966). The primary vectors for *Haemoproteus* parasites are biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) (Wirth 1974; Harwood and James 1979; Kettle 1982) and louse-flies (Diptera: Hippoboscidae) (Bequaert 1954; Kettle 1982). Avian *Plasmodium* is transmitted most commonly by *Culex* mosquitoes (Forrester et al. 1980; Atkinson and van Riper 1991; Telford et al. 1997; Nayar et al. 1998). The parasite genera also differ in that *Plasmodium* undergoes asexual multiplication in the peripheral blood, whereas *Haemoproteus* does not. For this reason, it is thought that *Plasmodium* is the more dangerous parasite for birds; *Haemoproteus* is generally more abundant and exhibits a higher proportion of infected erythrocytes, but has fewer health effects on the host.

The vertebrate hosts examined by Apanius et al. (2000) included six passerine birds (order Passeriformes): *Saltator albicolis* (Streaked Saltator), *Elaenia martinica* (Caribbean Elaenia), *Coereba flaveola* (Bananaquit), *Loxigilla noctis* (Lesser Antillean Bullfinch), *Tiaris bicolor* (Black-faced Grassquit), and *Vireo altiloquus* (Black-whiskered Vireo). Here we examine only four of these species, since *S. albicolis* and *E. martinica* were found to be free of malaria parasites. The remaining four species are among the most abundant and widespread passerine birds in the Lesser Antilles. *C. flaveola*, *L. noctis*., and *T. bicolor* are members of the subfamily Emberizinae in the family Fringillidae, while *V. altiloquus* is a more distantly related member of the family Vireonidae (AOU 2000). The ranges of the four species extend across the entire Lesser Antillean archipelago, and all are found in a similar range of forest and second growth habitats.

Sampling

The Lesser Antilles include six major volcanic islands with high elevation and a broad range of environments from rain-

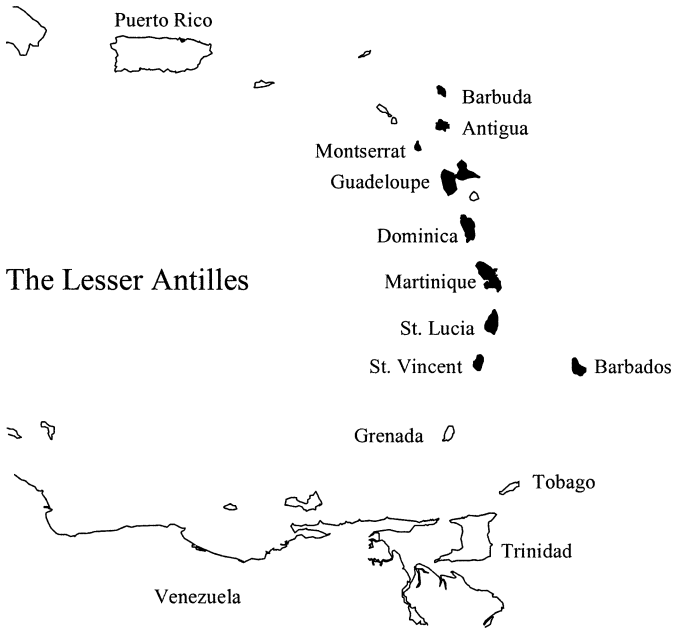


FIG. 2. Map of Lesser Antillean archipelago. Sampled islands are indicated by solid black fill.

shadow thorn scrub to montane cloud forests. From north to south these are Guadeloupe, Dominica, Martinique, St. Lucia, St. Vincent, and Grenada. A number of smaller volcanic islands and islands formed from uplifted marine sediments are distributed outside this core, including Barbuda, Antigua, and Montserrat. Barbados lies to the east of the main island chain (Fig. 2). Blood samples were collected from more than 600 individual birds of the four target species within nine islands in the Lesser Antilles (Appendix 1 and 2). Three of the islands (Dominica, Martinique, and St. Lucia) were sampled during July 1991 and additional samples from St. Lucia were collected in July 2000. The remaining six islands (Barbuda, Antigua, Montserrat, Guadeloupe, St. Vincent, and Barbados) were sampled during May 1993. Sampling sites were comparable between islands, and included low- to midelevation forest and second-growth habitats where all four species are found.

We caught birds using mist nets and took 5–10 μL of blood by wing or carotid venipuncture. Smears were prepared using ca. 2–3 μL of blood. Slides were air-dried, fixed in absolute methanol, and stained with Modified Giemsa Stain Solution (Sigma-Aldrich Corp., St. Louis, MO). The remaining blood sample was stored in buffer prior to DNA extraction. Samples collected in 2000 were stored in Puregene® cell lysis buffer (Gentra Systems, Minneapolis, MN). All others were stored at an ambient temperature in Queen's lysis buffer (Seutin et al. 1991). 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.

Molecular Analysis

DNA from blood samples in Puregene cell lysis buffer was extracted by salt precipitation according to the manufacturer's protocol. All other samples followed a standard phenol-chloroform extraction with dialysis in 1 X Tris EDTA buffer as described in Seutin et al. (1993). We amplified approximately 355 base pairs of the cytochrome *b* gene using the polymerase chain reaction (PCR). We designed two primers using published sequences of the cytochrome *b* gene for *Plasmodium* (Escalante et al. 1998): L15368 5′- AAA AAT ACC CTT CTA TCC AAA TCT-3′ and H15730 5′- CAT CCA ATC CAT AAT AAA GCA T-3′. The numbering of the primers refers to the approximate nucleotide positions within the human mitochondrial genome (Anderson et al. 1981). PCR reactions were run in 10 μl volumes that contained the following components in their final concentration: 2.5 mM MgCl, 0.2 mM of each dNTP, 0.4 μM of each primer and 0.5 units of *Taq* polymerase. One μl of the extracted DNA was used for amplification. Thermal cycling conditions were as follows: 1 min at 94°C, followed by 35 cycles with 0.5 min denaturation at 94°C, annealing at 48°C for 1 min., and elongation at 72°C for 1.5 min. After the 35 cycles, a final elongation step followed at 72°C for 3 min. We purified the amplified product by gel extraction. Sequencing was carried out on an automated sequencer (ABI Prism 377: Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. The cytochrome *b* sequences were edited and aligned using DNASTAR® software (Madison, WI) and are available through GenBank (accession numbers AY167239–AY167250).

Parasite lineages were identified based on genetic similarity; between lineage variation ranged from 2–11% uncorrected sequence divergence. Because these parasites have not been described, lineage boundaries were arbitrarily defined, but in all cases they exceeded two times the maximum level of intralineage variation. Values of cyt *b* sequence divergence as low as 1.0% have been observed between named malarial species of mammals (Escalante et al. 1998), and malarial species of lizards have been described at 3% sequence divergence (Perkins 2000). Within lineage variation was minimal, averaging 0.6% (approximately two base pairs), and did not provide additional detail regarding host and island distributions of lineages.

Infections for PCR positive individuals were confirmed by microscopy for the subset of samples collected in July 2001. In addition, the presence of both *Haemoproteus* and *Plasmodium* lineages, as identified by sequencing, was consistent with morphologically distinguishable characters found on blood smears. Multiple infections were identified by double peaks in sequence chromatograms, but by this criterion they were rare in this study. In cases in which lineage identity was unclear from a multiple infection, the sequence was removed from analysis.

Statistical Analysis

Because sample sizes were unbalanced between hosts and islands, we used contingency table analyses rather than analysis of variance to detect the influence of main effects and interactions on parasite prevalence (PROC CATMOD, SAS/STAT version 8.0, SAS Institute Inc., Cary, NC). Heterogeneity in parasite distributions was assessed by G-tests followed by partitioned analyses (Sokal and Rohlf 1994).

Results and Discussion

Overall Parasite Prevalence

We began our analysis by comparing the results of our expanded data set to those of Apanius et al. (2000). We sampled 621 individuals of the four species and detected 261 infections by PCR. Parasite prevalence showed no effect of the three sampling dates: July 2000, July 1991, and May 1993 ($\chi^2 = 1.71$, df = 2, $P = 0.43$). We therefore combined the parasite prevalence data from the three sampling periods. As in the Apanius et al. (2000) study, infections showed a strong species effect (Table 1). Parasite prevalence ranged from a high of 52% in *Loxigilla noctis* to 45% in *Coereba flaveola*, 35% in *Tiaris bicolor*, and 21% in *Vireo altiloquus*. In this study, islands demonstrated a marginal effect on parasite prevalence. However, a partitioned G-test analysis revealed that only one island (Antigua) contributed to the observed heterogeneity with slightly lower parasite prevalence ($G = 4.01$, df = 1, $P < 0.05$). The remaining locations showed no heterogeneity in overall infections ($G = 11.19$, df = 7, $P > 0.05$), implying that variation in parasite prevalence was not associated with characteristics of islands. A strong species-times-island interaction for variation in prevalence remained in our sample (Table 1). Thus, our results confirm, for a broader range of islands, previously described patterns for overall malarial parasite prevalence. Avian malaria shows a strong host effect, little to no island effect, and a significant host-times-island interaction, suggesting independent host-parasite interactions among island populations in the Lesser Antilles.

Infection by Parasite Lineage

We next separated the overall parasite infections into individual lineages by determining the malarial mtDNA cyt *b* sequence for 238 of the 261 infected individuals. Poor amplification or unclear chromatograms prevented us from including the remaining 23 infected individuals in subsequent sequence-based analyses. We identified 12 distinct parasite lineages (Fig. 3), but 98% of the infections could be assigned to one of eight common lineages sampled from multiple individuals and/or hosts. Half of the infections could be attributed to a single lineage, *Haemoproteus* C, which was recovered from all four species and all islands. Eight of the 12 malarial lineages were classified as *Haemoproteus* parasites based on their phylogenetic position (Bensch et al. 2000; Ricklefs and Fallon 2002); the remaining four were *Plasmodium*.

Effect of host species

The distribution of parasite lineages showed strong host species effects. Four of the eight primary lineages of parasites were found to infect *Coereba flaveola*; however, the two most abundant lineages (*Haemoproteus* C [HC] and *Plasmodium* C [PC]) made up 80% of this host's infections (see Fig. 3). Five parasite lineages were recovered from *Loxigilla noctis*, three of them (HC, *Haemoproteus* D [HD], and PC) accounted for 98% of infections. *Tiaris bicolor* was infected primarily by PC, while *Vireo altiloquus* was the only host species infected by *Haemoproteus* B [HB] and *Plasmodium* B [PB]. *V. altiloquus* also hosted three of the four unique lineages. In addition, parasite lineage PC, which was relatively common in the other host species, was conspicuously missing from *V. altiloquus*.

Table 1. Categorical model of the overall parasite prevalence with respect to hosts and islands.

Source	df	Chi-square	*P*
Intercept	1	33.86	<0.0001
Host	3	30.80	<0.0001
Island	8	15.43	0.0512
Host × Island	24	72.57	<0.0001

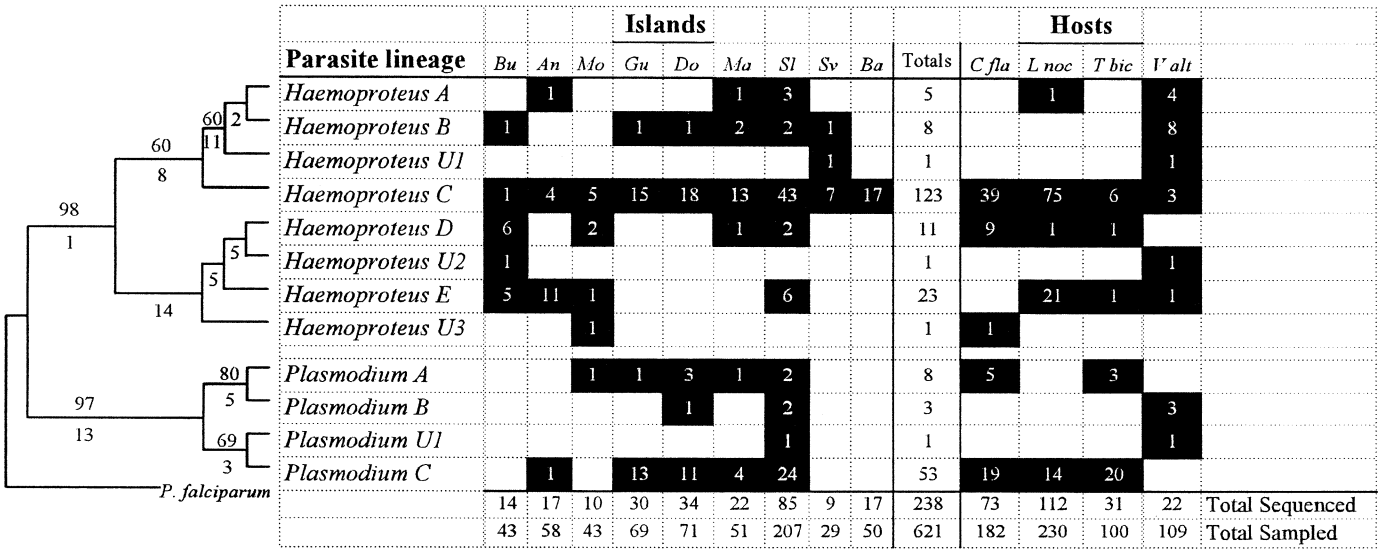
Host species clearly influence the prevalence of individual parasite lineages and of all lineages together. *Loxigilla noctis* and *Coereba flaveola* are more likely to be infected (44% on average), while *Tiaris bicolor* and *Vireo altiloquus* exhibit fewer infections (28% on average). Moreover, *V. altiloquus* hosts nearly half of the parasite lineages exclusively. Because these four host species live together in the same habitats, differences in exposure to infection are unlikely to cause the variation seen here. Although few data are available, individual lineages of avian malaria can be transmitted by several species of vector (Bennett and Fallis 1960; Garvin 1996). Assuming that each vector species is attracted to a variety of potential host species, routes of transmission are less likely to explain parasite specialization than do genetic differences between host species.

V. altiloquus is the most distantly related of the four host species. The fact that it also shares the fewest parasite lineages with the other hosts suggests that host effects in parasitism may be in part related to host phylogeny. Few studies to date have examined the evolutionary history of avian hosts with respect to their specific parasite lineages. Those that have, however, demonstrate some degree of host switching and sharing, but significant host conservatism among closely related parasite lineages, illustrating that host phylogeny indeed plays an important role in parasite distributions (Bensch et al. 2000; Ricklefs and Fallon 2002). Thus, host effects in the distribution of parasite lineages in the Lesser Antilles likely reflect a long evolutionary history of avian malaria in these four host species.

Effect of islands

Islands appear to have little to no effect on the distribution of overall parasite prevalence across nine islands. This result, while consistent with Apanius et al.'s (2000) findings, nonetheless seems striking given the amount of geographic heterogeneity found in other avian malaria studies (e.g. Freeman-Gallant et al. 2001; Valkiunas and Lezhova 2001). Variation in blood parasite prevalence, however, is often associated with environmental factors including temperature and elevational or longitudinal gradients that likely affect vector abundance (van Riper et al. 1986; Bennett et al. 1992; Schall and Marghoob 1995). Given the ecological similarity between the Lesser Antillean islands, an even distribution of parasites is not surprising.

While overall parasite prevalence was consistent across islands, individual parasite lineages varied considerably in



		Islands									Hosts				
Parasite lineage	Bu	An	Mo	Gu	Do	Ma	Sl	Sv	Ba	Totals	C fla	L noc	T bic	V alt	
Haemoproteus A		1				1	3			5		1		4	
Haemoproteus B	1			1	1	2	2	1		8				8	
Haemoproteus U1								1		1				1	
Haemoproteus C	1	4	5	15	18	13	43	7	17	123	39	75	6	3	
Haemoproteus D	6		2			1	2			11	9	1	1		
Haemoproteus U2	1									1				1	
Haemoproteus E	5	11	1				6			23		21	1	1	
Haemoproteus U3			1							1	1				
Plasmodium A			1	1	3	1	2			8	5		3		
Plasmodium B					1		2			3				3	
Plasmodium U1							1			1				1	
Plasmodium C		1		13	11	4	24			53	19	14	20		
	14	17	10	30	34	22	85	9	17	238	73	112	31	22	Total Sequenced
	43	58	43	69	71	51	207	29	50	621	182	230	100	109	Total Sampled

FIG. 3. Maximum parsimony tree for the 12 lineages of avian malaria parasites in the Lesser Antilles with their distributions among islands and hosts. Numbers above the branches indicate bootstrap support for 10,000 replications. Those below indicate number of substitutional changes for internal branches. The tree was rooted using a mammalian parasite, *Plasmodium falciparum*. Islands are arranged in geographic order from north to south. Common lineages are labeled *Haemoproteus* A–E and *Plasmodium* A–C. Unique lineages are labeled *Haemoproteus* U1–U3 and *Plasmodium* U1.

their geographic distributions. *Haemoproteus* parasites, as a group, were evenly distributed throughout the archipelago ($G = 4.50$, df = 8, $P = 0.8$), whereas *Plasmodium* parasites showed strong geographic structuring ($G = 51.06$, df = 8, $P < 0.001$) and were missing from three of the nine islands (Fig. 4). Furthermore, specific lineages within each parasite genus demonstrate significant heterogeneity across the archipelago (Table 2). Lineage *Haemoproteus* E [HE] for example, is more common on the northern islands and absent from most central islands, whereas lineages HC and PC are more strongly represented in the central islands, and are rare or absent in the north (see Fig. 3). The small sample sizes of the remaining lineages make their distributions difficult to assess statistically. Nonetheless, all of the common parasite lineages demonstrate some degree of patchiness across the archipelago.

Because the four host species occur abundantly throughout the Lesser Antilles, these varied geographic distributions suggest that parasites may be constrained in their ability to disperse to new locations and that parasite populations may disappear from islands. Geographic structuring and local extinctions of malarial parasites in lizards have also been reported for this region (Perkins 2001). However, as hosts, lizards and birds vary considerably in their abilities to disperse. Recent studies with wide geographic sampling have shown migratory birds can carry parasites between host breeding and wintering locations (Waldenström et al. 2002; Ricklefs et al. 2003). In spite of this, few of the Lesser Antillean parasite lineages described here have been detected in migratory birds in North America (R. E. Ricklefs et al., unpubl. ms.) and relatively few migrants pass through or winter in the Lesser Antilles. In addition, the resident avifauna apparently rarely disperse between islands, judging from the degree of genetic differentiation observed between island populations in many species (Ricklefs and Bermingham 1999). Thus, the constrained movement patterns of the hosts may contribute to the heterogeneous spatial distribution of avian malaria in the Lesser Antilles.

The geographic heterogeneity observed among Lesser Antillean malarial parasites indicates that interactions among parasite lineages might also influence distribution patterns.

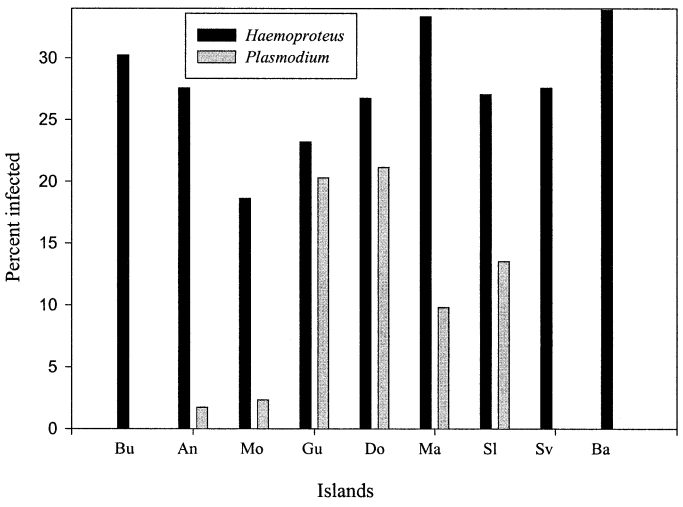


FIG. 4. Percent of individuals infected by *Haemoproteus* and *Plasmodium* parasites by island. Island names are abbreviated (see text for details) and arranged in geographic order from north to south.

TABLE 2. Heterogeneity of three lineages of parasites across the Lesser Antillean archipelago.

Source	df	*G*	*P*
Lineage HC	8	29.8	< 0.001
Lineage HE	8	41.7	< 0.001
Lineage PC	8	45.6	< 0.001

TABLE 3. Categorical model of the prevalence of lineage HC with respect to hosts and islands.

Source	df	Chi-square	*P*
Intercept	1	108.73	<0.0001
Host	3	39.21	<0.0001
Island	8	21.83	0.0052
Host × Island	24	81.40	<0.0001

Interactions between different malarial parasites within the same vertebrate host individual are poorly understood and have been described as positive (Richie 1988; Schall and Bromwich 1994), neutral (Staats and Schall 1996), and antagonistic (Richie 1988 and references therein). The observation that the prevalence of all malaria parasites together does not show an island effect (Table 1) while individual lineages do (Table 2), suggests that populations of malaria parasites interact with each other negatively. While overall prevalence of malaria parasites in a host population might reflect a general line of defense against *Haemoproteus* and *Plasmodium*, the relative abundance of particular parasite lineages within this framework may depend on competition between lineages, including cross-resistance to infection within individual hosts, and/or the match of specific genetic virulence and resistance traits in the parasite and host. These factors, accompanied by gene flow and local extinctions in parasite populations as well as over the phylogeographic history of the host, are likely to produce geographically heterogeneous parasite distributions and thus, host-parasite interactions.

Host-times-island interactions

A large sample size is required to detect a host-times-island interaction, such as that demonstrated for overall parasite prevalence. Lineage HC, which comprises just over half of all the infections in the four species of Lesser Antillean birds considered here, is the only lineage that lends itself to analysis of host and island effects with a categorical model. This analysis revealed both a species and island effect for lineage HC, and also demonstrated a significant host-times-island interaction (Table 3).

Other less well-sampled lineages also suggest host-times-island interactions. For example, lineage PC shows varying patterns of infection for three of the species across four of the central islands (Fig. 5). These patterns, similar to the one illustrated in Figure 1, demonstrate independent outcomes of host-parasite interactions in different island populations representing the same host species. Therefore, species- or island-specific attributes of particular avian malarial lineages do not explain the overall host-times-island interaction, given that they demonstrate the same level of independence in host infections.

The pattern of independent host–parasite interactions, then, is not explained by genetic differences in either the host populations (Apanius et al. 2000) or the parasite lineages at the mitochondrial level. The lability of infection patterns in this system suggests dynamic changes occurring more rapidly than the mtDNA substitution rate. Thus, if there is a genetic basis to the host-times-island interactions observed in this system, it is likely to be at a much finer scale of genetic differentiation than observed here. Therefore, further study of this phenomenon should include more rapidly evolving genetic markers that are under direct selection in the host–parasite interaction. Examples of such markers might include the major histocompatability gene loci in birds and genes that code for the parasite surface proteins that interact directly with the birds' immune systems (Hughes and Hughes 1995; Gilbert et al. 1998).

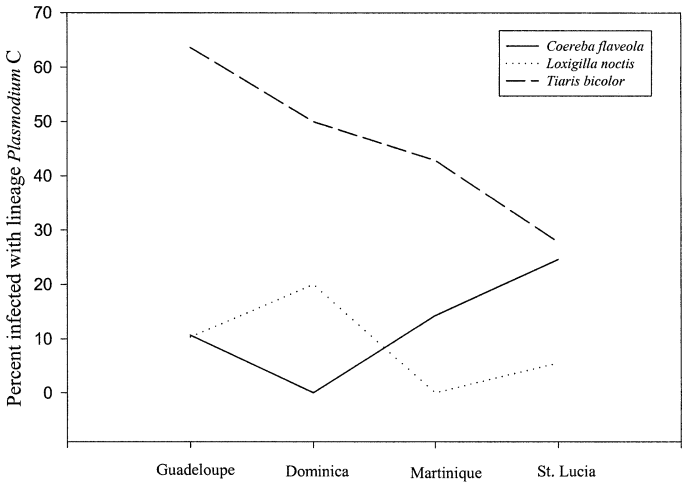


FIG. 5. Illustrative example of host-times-island interactions in prevalence of lineage *Plasmodium* C across four islands. (See also Fig. 1.)

Co-Evolutionary Dynamics in Avian Malaria

Avian malaria in the Lesser Antilles exhibits a low correlation among the outcomes (prevalence) of host-parasite interactions between island populations of the same species. This pattern suggests that at least some island populations of hosts and parasites co-evolve independently and that their interactions are dynamic and variable. Variation in species interactions, including host-parasite relationships, has engendered considerable interest in evolutionary ecology. An important synthesis of this interest has been the development of the geographic mosaic theory of co-evolution by Thompson (1994). Here we explore the extent to which this theory applies to our data and provide an additional explanation for the observed patterns of infection in this system.

According to the geographic mosaic theory, host-parasite associations may differ across their ranges due to geographically varying selection pressures in local environments and constantly changing genetic landscapes due to gene flow. When parasites are able to infect a number of hosts, the theory predicts a co-evolutionary pattern in which parasite populations alternate between hosts over evolutionary time to escape evolved host defenses (Thompson 1999). The model implies that host defenses effective against one parasite lineage are less effective against another and that no host population can be equally defended against all parasite lineages. By similar reasoning, no parasite lineage can be equally infective in all potential hosts. Alternation between hosts occurs locally and varies according to the community context of the interaction, which, according to the theory, may differ

over the geographic range of the parasites and their hosts. Co-evolutionary alternation then would provide a possible mechanism for the independence of host-parasite associations found in the Lesser Antilles.

Another prediction of the geographic mosaic theory is that transient mismatches, or maladaptations, will occur due to trait mixing across a variable interaction (Thompson 1999). Such mismatches may be present in our data as ''spill-over'' infections in which parasite lineages predominantly occurring in one host species are detected in low numbers in at least one other host, as seen in *Haemoproteus* lineages HD and HE (Figure 3). Therefore, our data exhibit a variety of patterns that are consistent with the geographic mosaic theory of co-evolution. However, the theory relies heavily on gene flow as well as community or habitat differences between populations. In the Lesser Antilles, gene flow between many adjacent island populations is weak or absent (Seutin et al. 1994; Bermingham et al. 1996; Hunt et al. 2001; Lovette et al. 1998, 1999a, 1999b; Ricklefs and Bermingham 1999) and habitat and community differences are not pronounced among the islands.

As an alternative, we suggest that the observed pattern of evolutionary independence in host-parasite interactions may be intrinsic to the interaction itself regardless of the local surroundings or gene flow from other localities. Chance mutations in either the host or its parasite could give rise to locally evolved, transient virulence-resistance relationships that would necessarily vary between isolated populations due to their random nature. Spill-over infections in this case would result from asymmetries in either virulence or resistance within the parasite and host species in a local community. Thus, variable malaria prevalence among different island populations of the same species in the Lesser Antilles is also consistent with the intrinsic evolutionary dynamics of the host-parasite relationship driven by mutation. Spatial variation in the community context of the parasite-host interaction may not be required to explain such patterns. It may be relevant that phases of population expansion and contraction of birds within the Lesser Antilles, identified through phylogeographic analysis, occur on time scales of 10^5–10^6 years (Ricklefs and Bermingham 1999, 2001), consistent with the appearance and time to fixation of favorable mutations within a population.

Whether our data represent a geographic mosaic of interacting communities or isolated evolutionary dynamics between hosts and their parasites will depend on the extent of gene flow between island populations, the scale of the co-evolutionary interaction, and the stability of host-parasite associations over time. Nonetheless, with respect to a widespread disease organism that offers an excellent host-parasite model system, the investigation of genetically distinct lineages of avian malaria provides a crucial first step in characterizing these co-evolutionary relationships. Indeed, as studies continue to reveal the finer partitioning of parasite lineages within host communities and across host geography, current concepts of host-parasite interactions may also need to evolve.

Conclusions

While overall malarial parasite prevalence in the Lesser Antilles does not appear to vary across geographically discrete locations, individual parasite lineages do vary. This pattern suggests that parasite lineages compete for infections, individual parasite lineages are constrained in their ability to move between locations, and parasite populations may disappear from individual islands. Also, there appears to be an upper limit to the number of host individuals that malaria parasites, as a community, can infect at any given time. This limit may be set by general defenses of hosts against malaria parasites. Within this general limit, variation in the relative frequency of different parasite lineages might reflect the different outcome of dynamic host-parasite co-evolution within a community setting.

Host species play a role in determining this variation; however, they do not explain it entirely. Hosts appear to be differentially susceptible to infection in general, and to individual parasite lineages. These differences reflect, to a certain extent, host phylogeny and thus, host-parasite co-evolution. Moreover, the significant host-times-island interaction for particular parasite lineages such as HC, permits the strong inference that the rate of host-parasite co-evolution exceeds our ability to detect it with our current mitochondrial markers of host and parasite evolution. In this system, patterns of association between birds and their malarial parasites appear to result from both the longer term evolutionary processes evidenced by host and parasite phylogenies, and shorter term interactions that might result from the co-evolutionary population genetic dynamics of host resistance and parasite virulence. Further characterization of this system will require research at the interface of ecological and evolutionary interactions by examining finer scale host and parasite responses to infections.

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APPENDIX 1.

Number of infected individuals and total sample size for the four host species across nine islands in the Lesser Antilles. Collection dates are also listed. Islands are arranged in geographic order from north to south. See text for complete island names. St. Lucia is listed twice by collection date.

Hosts	Islands									Totals
	Bu	An	Mo	Gu	Do	Ma	Sl	Sl	Sv	Ba	
C. flaveola	7/14	4/16	6/18	21/28	11/18	3/7	10/28	14/33	3/10	3/10	82/182
L. noctis	5/13	11/20	6/11	6/29	15/30	16/26	23/35	24/37	4/8	11/21	121/230
T. bicolor	0/14	1/18	2/10	8/11	7/10	3/7	4/9	6/9	1/5	3/7	35/100
V. altiloquus	2/2	1/4	0/4	1/1	4/13	2/11	5/26	5/30	1/6	2/12	23/109
Total	14/43	17/58	14/43	36/69	37/71	24/31	42/98	49/109	9/29	19/50	261/621
Date collected	May 1993	May 1993	May 1993	May 1993	July 1991	July 1991	July 1991	July 2000	May 1993	May 1993	

APPENDIX 2.
Distribution of parasite lineages by host and island. See Appendix 1 for host sample sizes.

Lineage	Host	Bu	An	Mo	Gu	Do	Ma	Sl	Sv	Ba	Totals
Haemoproteus A	*L. noctis*						1				1
	V. altiloquus		1					3			4
Haemoproteus B	*V. altiloquus*	1			1	1	2	2	1		8
Haemoproteus C	*C. flaveola*	1	4	1	13	9		5	3	3	39
	L. noctis			4	2	7	13	34	4	11	75
	T. bicolor							3		3	6
	V. altiloquus					2		1			3
Haemoproteus D	*C. flaveola*	6		1			1	1			9
	L. noctis							1			1
	T. bicolor			1							1
Haemoproteus E	*L. noctis*	5	10	1				5			21
	T. bicolor		1								1
	V. altiloquus							1			1
Haemoproteus U1	*V. altiloquus*								1		1
Haemoproteus U2	*V. altiloquus*	1									1
Haemoproteus U3	*C. flaveola*			1							1
Plasmodium A	*C. flaveola*				1	2	1	1			5
	L. noctis			1		1		1			3
Plasmodium B	*V. altiloquus*					1		2			3
Plasmodium C	*C. flaveola*				3		1	15			19
	L. noctis		1		3	6		4			14
	T. bicolor				7	5	3	5			20
Plasmodium U1	*V. altiloquus*							1			1
Totals		14	17	10	30	34	22	85	9	17	238