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Biogeographic patterns of avian malaria parasites in the Lesser Antilles: Prevalence, diversity, and community composition

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BIOGEOGRAPHIC PATTERNS OF AVIAN MALARIA PARASITES IN THE LESSER ANTILLES: PREVALENCE, DIVERSITY, AND COMMUNITY COMPOSITION

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ABSTRACT

We investigated determinants of local and regional species richness and community composition of avian malaria parasites (*Haemoproteus* and *Plasmodium*) in the Lesser Antilles. Chapter 1 addresses the local parasite richness and community structure, or lack thereof, on Barbados. We found only two parasite lineages on this island, one of which was recovered from only 2 birds, which stands in stark contrast to the much higher diversity on other islands. In addition, we investigated what factors may explain the absence of avian malaria in southeastern Barbados, and found this area to be drier, warmer, and supporting less vegetation. Chapter 2 addresses parasite diversity, primarily beta diversity, in the Lesser Antilles. In this chapter, we investigated the role of host history and compound communities in structuring local ensembles. We found that host genetic distance does not correlate with ensemble dissimilarity but that more phylogeographically structured host species exhibit more unique parasite ensembles compared to the compound community than do hosts that are not phylogeographically structured, suggesting that host history does influence parasite ensembles.

Keywords: Avian malaria, *Haemoproteus coatneyi*, environmental variation, beta diversity, island biogeography, Lesser Antilles

CHAPTER 1: LOW DIVERSITY AND HIGH INTRA-ISLAND VARIATION IN PREVALENCE OF AVIAN MALARIA PARASITES ON BARBADOS, LESSER ANTILLES

L. Maria E. Svensson and Robert E. Ricklefs

ABSTRACT

We screened common bird species on Barbados for avian malaria parasites to determine whether the apparent absence of avian malaria parasite diversity found in previous studies could be due to a small sample. After screening the birds, we found that avian malaria parasites were absent from the southeast, whereas they were abundant in several host species in the northwest. Therefore, we also investigated environmental and host population genetic differences between the parasite-free and the parasite-afflicted regions. Sixty-two out of 257 birds were infected with avian malaria parasites on Barbados in 2007. Fifty-seven of the infections were identified as lineage HC, the only lineage recovered in the previous study. Two of the infections were identified as lineage HD, a lineage prevalent in *C. flaveola* on Grenada. We discuss the possibility of infrequent colonization events and absence of vectors as explanations for Barbados's low avian malaria parasite diversity. We found no host genetic differences but striking environmental differences between the parasite-free and the parasite-afflicted regions. The southeast is warmer and drier than the northwest. The southeast also supported less vegetation than the northwest in one of two years analyzed. We discuss the influence this harsher environment may have on vector survival.

INTRODUCTION

The biogeography of malaria parasites in wildlife communities has been studied both on continents and islands (*e.g*. Greiner *et al*., 1975; Bennett *et al*., 1992; Staats and Schall, 1996; Apanius *et al*., 2000; Perkins, 2001; Ricklefs and Fallon, 2002; Bensch and Åkesson, 2003; Fallon *et al*., 2003a; Fallon *et al*., 2005; Gibb *et al*., 2005; Beadell *et al*., 2006; Durrant *et al*., 2006). Among other things, these studies have demonstrated that distributions of parasites are often patchy in island systems, and that diversity of malaria communities can vary greatly between locations. The lower prevalence or complete absence of particular parasite lineages in some locations may be explained by competition between lineages (*e.g*. Bensch and Åkesson, 2003; Fallon *et al*., 2003a, 2005; Bensch *et al*., 2007), by evolved resistance to the parasites (*e.g*. Wakelin and Apanius, 1997), by low host density, which will limit the resources available for parasites (*e.g*. Price, 1990), as a result unsuccessful dispersal (Paterson and Gray, 1997), by local extinctions (Fallon *et al*., 2005), or by unfavorable environmental conditions for parasite transmission (Freed *et al*., 2005) and/or vector survival (*e.g*., Mellor *et al*., 2000). For example, low prevalence of vector-born parasites in arid regions (*e.g*. Bennett *et al*. 1992; Little and Earle, 1995; Tella *et al*., 1999; Valera *et al*., 2003) and on islands compared to the nearby mainland (Super and van Riper, 1995) has been documented, which suggest that environmental variation in climate, vegetation, and dispersal may explain the absence of avian malaria parasitism in certain areas. This study focuses on the avian malaria parasite community on the island of Barbados, which was found in an earlier study to harbor a single parasite lineage with a broader host distribution compared to other islands in the Lesser Antilles, where the avian malaria parasite community is more

diverse (Fallon *et al.*, 2005). Additional sampling in this study confirms the low diversity of the Barbados parasite community and also revealed a striking heterogeneity in parasite prevalence between areas on the same island.

In the Lesser Antilles, avian malaria parasite communities exhibit high cross-island variation in lineage diversity. In general, the number of parasite lineages defined by variation in mitochondrial gene sequences ranges from 5 on Barbuda, a low limestone island in the north of the archipelago to 13 on St. Lucia, a high volcanic island in the south (Fallon *et al.*, 2005). A survey of avian malaria parasite diversity in the Lesser Antilles recovered only one lineage (*Haemoproteus* sp. haplotype C; HC, GenBank Accession AY167242) from Barbados (Fallon *et al.*, 2005). The prevalence of HC on Barbados (25%), however, was similar to the average avian malaria parasite prevalence of 28% across 10 different islands. HC is the most abundant parasite lineage in the Lesser Antilles (195 recoveries; making up 44% of all infections), has a wide host breadth (24 host species) and a broad geographic distribution (13 islands). On Barbados, HC was recovered from 5 species, two of which were not infected with HC on other islands (*Quiscalus lugubris* and *Columbina passerina*), suggesting that this parasite lineage exploits a wider range of hosts on Barbados (Fallon *et al.*, 2005).

Barbados (Figure 1), unlike the other Lesser Antillean islands, is geologically young (~1 million years compared to the other islands' ages of 20-30 million years), dry, coralline as opposed to volcanic, and has never supported tropical forests (Speed, 1994; Lovette *et al.*, 1999; Buckley and Buckley, 2004). The eastern part of Barbados is characterized by strong winds and stunted woody vegetation (Randall, 1970; Buckley and Buckley, 2004). Compared to the other Lesser Antillean islands, Barbados has few

resident species of birds. Although as many as 42 non-raptorial land bird species occur on a single island (St. Lucia), and 65 species occur throughout the Lesser Antilles (Raffaele *et al*., 1998), Barbados is home to only 23 resident species (Buckley *et al*., 2008). However, some of the most abundant species in the archipelago (*e.g*. *Coereba flaveola, Tiaris bicolor,* and *Q. lugubris*) are also abundant on Barbados. *Loxigilla barbadensis* is an endemic species to Barbados (Buckley and Buckley, 2004) and is closely related to the widespread and abundant *Loxigilla noctis* (Lovette *et al.* 1999).

We sampled additional individuals on Barbados in 2007 to determine whether the apparent absence of avian malaria parasite diversity could be due to the small sample in 1993 (n=85). Avian malaria parasite diversity is high on other islands in the Lesser Antilles, and it is surprising that additional parasite lineages did not reach Barbados with colonizing birds.

An interesting finding during our survey was the absence of avian malaria parasites on the southeastern coast of Barbados, which led us to do additional analyses to investigate two possible reasons for this pattern: (1) whether southeastern host populations are genetically different from the northwestern host populations, which would suggest that the southeastern population has evolved in isolation long enough to possibly have evolved resistance to the parasites, and (2) whether environmental variables and vegetation differ significantly between the sample areas.

METHODS

We sampled four locations on the island (Figure 1; Table 1); one on the southeast (windward) side (St. Martin, Parish of St. Philip), one in the middle/north (Turners Hall

Woods, Parish of St. Andrews), and two on the west side (leeward; Holetown and Trents, Parish of St. James). The habitats were different between the net sites. St. Martin was heavily developed and had no forest. Turners Hall Woods had dry forest with interspersed grazing patches for cattle and sheep, was higher in elevation (185 m) than the other locations (8-65 m), and relatively undeveloped. The Trents locality consisted mainly of grazing patches with interspersed clumps of trees. The Holetown sites were in the backyard of the Bellairs Research Institute (ground-trapping only) and in the Coral Reef Club hotel garden, which consisted of lush green vegetation and flowering bushes.

Birds were captured in mist nets or in ground traps that were baited with cooked rice and seeds, during the months of May and June in 2007. Nets were typically opened by 6:30 am and closed by 11 am. We included an additional 15 samples of *Q. lugubris* from Holetown, which were obtained during the same year by K. Monceau at the Université de Bourgogne, Dijon, France.

Five to ten μL of blood was obtained via brachial venipuncture for each bird individual. Four drops of blood were spread onto a glass slide, which was subsequently air dried and fixed for one minute in absolute methanol. Slides were stained with Giemsa, either using a differential rapid bloodstain solution kit (EK Industries, Inc., Joliet, IL) following the manufacturer's protocol or following the protocol of Valkiunas (2005). Remaining blood was placed in 300 μL of lysis buffer (0.1 M Tris, pH 8.0; 0.1 M EDTA, pH 8.0; 0.01 M NaCl; and 2.5% SDS). The entire 300 μL solution was used when extracting DNA by the following procedure. First, 10 μL of Proteinase K (10 mg/mL) was added and the mixture was incubated at 55°C overnight. Proteins were precipitated by adding 100 μL Puregene ammonium acetate precipitation (Gentra

Systems, Inc., Minneapolis, MN) to the sample, vortexing it for 20 sec, and spinning it at maximum speed for 3 min in a Spectrafuge 24D (Labnet International, Inc., Woodridge, New Jersey) microcentrifuge. The pellet was discarded and DNA was precipitated by adding 300 μL of isopropanol (100%) at 0°C, turning the sample on end 50 times, and spinning at maximum speed for 1 min in a microcentrifuge. The DNA pellet was washed with 300 μL 70% ethanol, dried, and eluted with 1X TE buffer.

We wished to determine whether the avian host populations were genetically differentiated across the island and sequenced the cytochrome *b* gene of the hosts using primers L14990 (Kocher, 1989 in Helm-Bychowski and Cracraft, 1993) and H16065 (Helm-Bychowski and Cracraft, 1993). For *C. flaveola*, 24 individuals (7 from SE and 17 from NW) and 966 bp were analyzed. For *L. barbadensis*, 25 individuals (11 from SE and 13 from NW) and 994 bp were analyzed. We created parsimony haplotype networks in TCS v.1.21 (Clement *et al.*, 2000). The TCS program produces a statistical parsimony network using methods described by Templeton *et al.* (1992; Clement *et al.*, 2000).

Infection status of 257 birds was determined by PCR, following the protocol of Fallon *et al.* (2003b), which amplifies a segment of mitochondrial ribosomal RNA-encoding DNA. The presence of a 153 bp long band scored an individual as positive for *Haemoproteus* and/or *Plasmodium*. In addition, a second screening of *Haemoproteus*/*Plasmodium* infections was performed by amplifying the parasites' cytochrome *b* gene, following the *cytb*/outer protocol of Martinsen *et al.* (2007). All samples screened using the latter set of primers also amplified using the former set. Those samples that scored positive but did not amplify using the *cytb*/outer primers used in Martinsen *et al.* (2007) were amplified using either primer set 413F (5'-GTG CAA

CYG TTA TTA CTA A-3') and 926R (5'-CAT CCA ATC CAT AAT AAA GCA T-3'; Ricklefs *et al*., 2005) or the *cytb*/nested protocol of Martinsen *et al*. (2007). Each of the primers in the 413F-926R set were used at 200 nM concentrations along with 200 nM dNTP, 1X buffer, 2 μM $MgCl_2$, and 1.25 units of TaKaRa Taq™ (TaKaRa Bio Inc., Shiga, Japan) in a 50 μL reaction. The PCR protocol follows that of Ricklefs *et al.* (2005).

The likelihood ratio statistic (equivalent to the *G* statistic) was used in JMP™ 5.0 (SAS Institute Inc., Cary, NC) to determine significant differences in malaria parasite prevalence between sites. The whole or part of the cytochrome *b* gene was sequenced for parasite haplotype identification. All sequencing was performed on a 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences were edited and aligned in SeqMan™ II 4.0 (DNASTAR Inc., Madison, WI). G. Valkiunas assigned one of the mitochondrial haplotypes we recovered to a morphological species using one *L. barbadensis* and two *C. flaveola* blood smears. Morphological characters used for identification include number of granules present, shape, attachment to the host cell nucleus and/or host cell wall, and the nucleus position of the parasite (see Valkiunas, 2005).

In DIVA-GIS (http://www.diva-gis.org/), we used climate data at a ~ 1 km resolution from the WorldClim database (Hijmans *et al*., 2005) to characterize the environments of the northwestern area, where avian malaria parasites were present, including Holetown, Trents, and Turners Hall Woods, and the southeastern area, where avian malaria parasites were absent, including St. Martin. The WorldClim database provides interpolated climate surfaces of monthly averages of temperature and

precipitation between years 1950 and 2000 measured from weather stations (Hijmans *et al*., 2005).

To determine whether our sampling areas could be differentiated by climate, we created 300 random points in ArcGIS v9.2 (ESRI, Redlands, CA) and selected 40 points each from the northwestern and southeastern regions. None of these points had identical coordinates, but they might have been in the same grid cells. We exported data of 19 climate variables (each of which is a measure either of temperature or precipitation) for each point from DIVA-GIS. To determine whether it is possible to discriminate between the malaria parasite-afflicted region in the northwest and the malaria parasite-free region in the southeast using climate data, we performed a discriminant analysis in SPSS version 13.0 (SPSS, Inc., Chicago, IL).

RESULTS

Of the 257 birds screened for avian malaria parasites, 62 (24.1%) were infected (Table 1). This prevalence is similar to that obtained in 1993 (25%). Only four of the 11 species sampled yielded infections. Samples of several other species were small: 1 *Molothrus bonariensis*, 6 *Zenaida aurita*, and 9 *Tyrannus dominicensis*. With a prevalence of 0.25, the probability of detecting no infections in a sample of 9 individuals is 0.075. However, screening of 140 additional *Z. aurita* did not yield any infected individuals (K. Monceau, pers. comm.). All 17 *T. bicolor*, 17 of 56 *C. flaveola* (30.4%), 27 of 72 *L. barbadensis* (37.5%), and one of 12 *Vireo altiloquus* (8.3%) were infected. None of the 46 birds screened on the southeastern side of the island were infected. These included 10 *C. flaveola* and 11 *L. barbadensis*. In Holetown, the prevalence in *C. flaveola* and *L.*

barbadensis was 57.3% (13 of 39) and 39.5% (15 of 38), respectively. In Trents, prevalence in the two species was 100% (2 of 2) and 45.5% (5 of 11), respectively. In Turners Hall Woods, the prevalence was 40.0% (2 of 5) and 58.3% (7 of 12) respectively. When *C. flaveola* and *L. barbadensis* from Turners Hall Woods, Trents, and Holetown were combined into a single "parasite-afflicted" region, the total prevalence was 41.1%. At this level, one would have expected 8 of the 21 sampled *C. flaveola* and *L. barbadensis* from St. Martin to be infected. The absence of avian malaria parasites in the southeast is significant compared to the combined prevalence in the northwest sites (G=19.8, df=1, P<0.0001).

We successfully obtained 42 parasite cytochrome b sequences of at least 983 bp and an additional 17 sequences >450 bp from three host species (*L. barbadensis*, n=26; *C. flaveola*, n=16; and *T, bicolor*, n=17). Fifty-seven of these sequences (97%) were identical and identified as lineage HC (GenBank Accession AY167242). The longer fragments we obtained differ by only one nucleotide from *H.* sp. haplotype 31 (GenBank Accession AF465579). Two sequences (3%) recovered from *C. flaveola* were identified as lineage *Haemoproteus* sp. haplotype D (HD; GenBank Accession AY167243), which is synonymous to *H.* sp. haplotype 6 (GenBank Accession AF465567). We were unable to sequence the single infection from *V. altiloquus*. Lineage HC was identified by G. Valkiunas as *Haemoproteus (Parahaemoproteus) coatneyi.*

The haplotype networks for both *L. barbadensis* and *C. flaveola* showed no intra-island structure of mtDNA: the two regions share the most abundant host cytochrome *b* haplotypes (Figure 2).

Of the 19 climate variables included in the discriminant analysis, six failed the tolerance test (a measure of redundancy), and the thirteen included variables are shown in Table 3. The discriminant analyses separated the two regions completely (Wilks' lambda=0.041, χ^2=228.4, p<0.001, df=13), recognizing, however, that the samples of random localities were highly pseudo replicated. Nonetheless, it is clear that the malaria parasite-free, southeastern region is warmer, drier, and exhibits greater variation in both temperature and precipitation throughout the year than the malaria parasite-afflicted, northwestern region (Table 3).

DISCUSSION

Haemoproteus (Parahaemoproteus) coatneyi

H. coatneyi (lineage HC of Fallon *et al.* 2005 and lineage OZ21 of Ricklefs *et al.* 2005) has been recorded from several emberizid passerine species from North America and the West Indies including *C. flaveola* (Valkiunas, 2005). This is the first recognition that the parasites of *L. barbadensis* and *T. bicolor* on Barbados are *H. coatneyi*. *H. coatneyi* is synonymous with *H. coereba*, *H. paruli*, and *H. thraupi* (Valkiunas, 2005). We do not know whether additional mitochondrial lineages also would be considered the same morphological species *H. coatneyi*. Because of poor blood smear quality, we were unable to identify the closely related mitochondrial sister lineage to HC, HH (which is 1.8% different in its cytochrome *b* sequence from HC) to its morphological species. Because previous biogeographic studies on avian malaria parasite in the Lesser Antilles discuss the distribution of mitochondrial lineages as opposed to morphological species,

and because we are yet uncertain of species limits in avian malaria parasites in the Lesser Antilles, we will hereafter refer to lineage HC instead of *H. coatneyi*.

Diversity and distribution

With 78 *Haemoproteus* sp. cytochrome *b* sequences from Barbados (19 from 1993 and 59 from 2007), of which 76 were HC and only 2 were HD, we conclude that Barbados supports a much reduced parasite fauna compared to other Lesser Antillean islands sampled to date. Because HC is the most common avian malaria parasite elsewhere in the Lesser Antilles, it would be the most likely lineage to successfully establish on Barbados. HD is rare on most islands and in most species, except on Grenada, where it is the predominant lineage in *C. flaveola* (Fallon *et al.*, 2005).

The low parasite diversity on Barbados could have resulted from a failure of additional lineages of parasites to colonize the island or the extinction of previously established lineages. Fallon *et al.* (2005) reasoned from the prevalence of several parasite lineages on the probable source islands of St. Vincent and St. Lucia that additional lineages must have arrived with avian colonists and either failed to become established or went extinct. The youth of the island and its peripheral location to the other islands also would favor unsuccessful colonization by other parasite lineages. Vector-borne parasites are more likely to disperse via their vertebrate hosts, in which infection prevalence is relatively high, than their smaller invertebrate vectors, which exhibit much lower infection prevalence (Lehane, 1991; Gager, 2008). Finding that all bird species on Barbados trace back to a single founding mtDNA lineage per species, Lovette *et al.* (1999) concluded that small land birds disperse to Barbados infrequently on an

evolutionary scale. This implies that there have been few opportunities for parasites to colonize Barbados via their vertebrate hosts.

Also, failure of different parasite lineages to colonize Barbados may be the result of an absence of appropriate vectors. For example, the absence of vectors is thought to underlie the much-reduced prevalence of avian haematozoa on San Miguel Island as compared to the California coast (Super and van Riper III, 1995). Data collected by Belkin and Heinemann (1976) show that 3 potential vector species of mosquito (*Aedes [Howardina] busckii*, *Anopheles [Nyssorhynchus] aquasalis* Curry, *Culex [Melanoconion] idottus* Dyar) are absent from Barbados while present on Dominica, St. Lucia, and Grenada, where the common *Plasmodium* lineage PC has been recovered (Table 4). *Aedes (Howardina) busckii* is the most abundant of the three, and it is not know to be a human-biting mosquito (Stone, 1969). In addition, the genus *Anopheles* is completely absent from, and the genus *Weyomyia* was recovered from only one locality on Barbados, while present on Dominica, St. Lucia, Grenada, and St. Vincent, where *Plasmodium* is present. However, *Culex quinquefasciatus*, which is known to vector avian malaria parasites in Hawaii (Warner, 1968), is abundant on Barbados (Table 4). Potential vector species are considered those belonging to a potential vector genus listed in Valkiunas (2005, p. 134).

It remains to be determined how frequently a single infected colonist can lead to the establishment of a new parasite lineage on an island. This would depend largely on the potential host breadth of the parasite and the diversity of other potential hosts on the island. Lineage HC infects a large number of bird species, and the arrival of a single HC infection likely would immediately have large numbers of potential hosts to infect. More

specialized parasite lineages would lack this potential, relying on an initial rapid increase in the colonist population to become established.

The widespread distribution of HC in the Lesser Antilles and its derived as opposed to basal position in the cytochrome *b* phylogeny (Fallon *et al*., 2005), suggest that it has spread throughout the archipelago recently. *T. bicolor*, *C. passerina*, and *Q. lugubris* are the most recent arrivals to Barbados because they are genetically identical to their source populations at the mtDNA ATPase 6 and 8 locus (Lovette *et al*., 1999). Based on genetic relationship, *T. bicolor* and *C. passerina* apparently colonized Barbados from St. Lucia, whereas *Q. lugubris* colonized Barbados from Trinidad (Figure 1). On St. Lucia, *T. bicolor* has a higher prevalence of a *Plasmodium* lineage (PC; 50% of infections) than of HC (30% of infections); St. Lucia is the only island other than Barbados where HC is known to infect *T. bicolor* (unpublished data). Remarkably, all *T. bicolor* individuals sampled on Barbados in 2007 were infected with HC (n=17). On other islands, the prevalence of malaria parasites in *T. bicolor* ranges from 6% on Antigua to 73% on Guadeloupe (Fallon *et al*., 2005). On islands closest to Barbados (*i.e*., St. Lucia, St. Vincent, and Grenada), avian malaria prevalence in *T. bicolor* ranges from 13% on Grenada to 56% on St. Lucia (Fallon *et al*., 2005). In our 2007 sample from Barbados, individuals of *C. passerina* and *Q. lugubris* were not infected with avian malaria parasites. In 1993, however, one of 5 *C. passerina* and one of 14 *Q. lugubris* were infected by lineage HC. Moreover, *C. passerina* and *Q. lugubris* are both infected with parasite lineages other than HC on their source-islands. In fact, HC has not been recovered from either *Q. lugubris* or *C. passerina* elsewhere (Fallon *et al*., 2005). Thus, if HC colonized recently, it did so either within a host species that has subsequently rid

itself of lineage HC both on Barbados and throughout the rest of the Lesser Antilles, or within *T. bicolor*, in which it has subsequently reached much higher prevalence than in the host's source populations.

Intra-island parasite prevalence

The absence of avian malaria parasites in the southeastern region of Barbados could be due to any one or a combination of several factors: (1) evolved host resistance to the parasites (*e.g.* Ricklefs, 1992; Wakelin and Apanius, 1997), (2) evolved vector resistance to the parasites (*e.g.* Collins *et al.*, 1986), (3) low host density (*e.g.* Price, 1990), or (4) the absence of appropriate vectors (*e.g.* Bennett *et al.*, 1992). We could not investigate either host or vector resistance directly, but mitochondrial data from *L. barbadensis* and *C. flaveola* show that the northwestern and southeastern populations have not been separated long enough for location-specific cytochrome *b* haplotypes to evolve. It is possible that they could have evolved resistance to avian malaria parasites even though they have not been separated long enough to show mitochondrial differentiation; nevertheless, the shared mitochondrial haplotypes between the southeast and northwest suggests that *L. barbadensis* and *C. flaveola* may be panmictic across Barbados. St. Martin is closest to Turners Hall Woods of the malaria parasite-afflicted localities (19 km) and farthest from Holetown (22 km). The three parasite-afflicted regions are within 7 km of each other. The habitat between the parasite-afflicted and parasite-free regions is mostly agricultural; however, *C. flaveola* and *L. barbadensis* are often found in gardens and among human settlements, and it is unlikely that agricultural landscapes or the ~20

km distance would create a significant barrier between populations of these two species on an evolutionary time-scale.

The *C. flaveola* and *L. barbadensis* populations appeared to be denser in Holetown (137 captures per 100 net-hours) than any other location. However, host density was lower in Turners Hall Woods and Trents, where parasite prevalence did not differ from that in Holetown, than in St. Martin; thus, host density does not explain the absence of avian malaria parasites in the southeast. We caught 24 *C. flaveola* and *L. barbadensis* per 100 net-hours in St. Martin, 16 per 100 net-hours in Turners Hall Woods, and 9 per 100 net-hours in Trents. Although mist net capture data might provide biased estimates of host density (*e.g.* Remsen and Good, 1996), they undoubtedly reflect general trends in numbers provided that habitat, weather, and time of day are reasonably matched.

The result of the discriminant analysis of 13 climate variables suggests that the southeastern region is warmer and drier than the northwestern region (Table 3), which is concordant with what Randall (1970) reported. We also compared vegetative cover between the two regions, as measured by the normalized difference vegetation index (NDVI) in 2000 and 2001 (methods available upon request). We found that whereas there was no difference in vegetation cover in 2000 (SE NDVI = 0.13±0.22 and NW NDVI = 0.l9±0.15 [mean ± standard deviation]; U=888.5, p=0.39), the index was lower (which means that there was less vegetation cover) in the southeast (NDVI = 0.04±0.14) than in the northwest (NDVI = 0.18±0.21) in 2001 (U=1227.5, p<0.001).

These environmental differences between the regions could explain why the northwest harbors avian malaria parasites whereas the southeast does not. *Haemoproteus*

sp. may be sensitive to the ambient environment because much of their life cycle takes place in an ectotherm vector. Although no studies have investigated the influence of the environment on *Haemoproteus*, *Plasmodium* has demonstrated sensitivity to ambient temperature. Freed *et al.* (2005) attributed the absence of avian *Plasmodium* at high elevations in Hawaii to low temperatures that prevent malaria development in mosquitoes. Cool temperatures would not be a problem on Barbados, where temperatures range from 18 to 32°C throughout the year.

More likely, the harsher environment on southeastern Barbados affects vector communities. Biting midges in the genus *Culicoides*, the suspected vectors of *Haemoproteus* sp. (Valkiunas, 2005; Mullens *et al.*, 2006), are highly influenced by climatic conditions throughout their lifecycle (Mellor *et al.*, 2000). For example, larva exhibit higher mortality rates under hot conditions (Mullens and Rodriguez, 1992, reviewed in Mellor *et al.*, 2000), and adults exhibit a higher mortality rate under hot and windy conditions (Hunt *et al.*, 1989; Wellby *et al.*, 1996; reviewed in Mellor *et al.*, 2000). Rawlings *et al.* (2003), found a significantly lower (but not non-existent) prevalence of *Culicoides* in dry, arid, and cold climates. However, they did find high prevalence of *Culicoides* in their dry, semi-arid, and hot areas, which would probably most closely resemble Barbados's southeastern region. Purse *et al.* (2004) found that some *Culicoides* species appear more moisture dependent than others and that prevalence of some species is higher in cooler areas. In sum, dry, hot, and windy conditions, which characterize the southeastern region of Barbados, have been shown to be unfavorable to several *Culicoides* species.

Little is known about avian biting midges in the Caribbean region. Surveys on mammalian biting midges, however, suggest that they are abundant, but that the diversity is lower on Barbados compared to many other West Indian and Central American regions (Greiner *et al*., 1993). Evidence from the distribution of mosquitoes, although not suspected vectors of *Haemoproteus*, show that the most abundant mosquito species, *Culex (Culex) nigripalpus* Theobald is present both in the northwest and southeast regions on Barbados (Belkin and Heinemann, 1976); however, there are fewer records from the parish of St. Philip than from most other parishes on Barbados. This might be the result of uneven sampling effort, but it might also reflect reduced mosquito abundance in the southeast. An important next step in avian malaria parasitism research of this kind on Barbados would be a survey of the potential vector communities.

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TABLES

Table 1. List of localities sampled on Barbados in 2007. Total catch includes birds not sampled (*i.e.*, hummingbirds). Net hours do not include ground-trap effort. Coordinates were obtained from Google Earth (2008, Europa Technologies).

Locality	Coordinates	Elev (m)	Sites	Days	Date range	Net Hours	Total Catch
St. Martin	13.09415 N;	45	2	4	25-May;28-May	92.6	48
Turners	13.22791 N;	185	1	3	30-May;01-Jun	106.5	44
Trents	13.19167 N;	65	1	3	02-Jun;05-Jun	154.2	35
Holetown	13.19153 N;	8	3	6	02-Jun;05-Jun	58.5	137

Table 2. Number of individuals of host species sampled, infected individuals, successfully sequenced individuals, and prevalence in each infected species.

Family	Species	Infected	Sampled	Prevalence	Sequenced
Columbidae	*Zenaida aurita*		6		
Columbidae	*Columbina passerina*		18		
Emberizinae	*Loxigilla barbadensis*	27	72	0.375	26
Emberizinae	*Coereba flaveola*	17	56	0.304	16
Emberizinae	*Tiaris bicolor*	17	17	1.000	17
Icterinae	*Quiscalus lugubris*		17		
Icterinae	*Molothrus bonariensis*		1		
Parulinae	*Dendroica petechia*		16		
Tyrannidae	*Ealenia martinica*		33		
Tyrannidae	*Tyrannus dominicensis*		9		
Vireonidae	*Vireo altiloquus*	1	12	0.083	
TOTAL		62	257	0.241	59

Table 3. Average climatic values from 40 random points per region (northwest [NW] and southeast [SE]). T = temperature. Variables are described in the text. Temperature variables are measured in degrees Celsius and precipitation variables are measured in millimeters. NW ± and SE ± are standard deviations. An asterisk denotes significant differences between group means. More detailed description of the variables can be found at www.worldclim.com.

Climate variable	NE	NW ±	SE	SE ±
Annual mean T*	25.43	0.46	25.87	0.23
Mean diurnal T range*	8.38	0.07	8.91	0.09
Isothermality (in percent)	76.06	0.41	76.10	0.48
T seasonality (in percent)*	88.84	1.47	92.76	2.51
Max T of warmest month*	30.42	0.43	31.19	0.29
Mean T of wettest quarter*	25.89	0.45	26.40	0.23
Annual Precipitation*	1389.10	49.36	1268.85	35.88
Precipitation of the wettest month*	191.38	6.41	177.23	2.57
Precipitation of the driest month*	42.45	1.72	39.93	1.29
Precipitation seasonality*	48.72	1.45	49.26	0.76
Precipitation of the driest quarter*	150.30	9.83	137.13	5.32
Precipitation of the warmest quarter	434.85	17.44	439.98	28.01
Precipitation of the coldest quarter*	233.00	8.60	217.68	6.07

Table 4. Distribution and abundance of Lesser Antillean mosquito species. All species from Barbados (BA; boldface), but only species with more than 10 records combined between the islands of Dominica (DO), Saint Lucia (SL), Saint Vincent (SV), and Grenada (GD) are included. Species found in the Parish of St. Philip are marked with an asterisk following the number of individuals identified.

Species	DO	SL	SV	BA	GD
Aedes (Howardina) busckii	22	30			18
Aedes (Ochlerotatus) taeniorhynchus		15		8*	16
Aedes (Ochlerotatus) tortilis		18			
Aedes (Stegomyia) aegypti	7	3	1	2	8
Anopheles (Nyssorhyncus) aquasalis Curry	7	22			7
Anopheles (Nyssorhyncus) argyritarsis Robineau-Desvoidy	21	8	5		18
Choretrella appendiculata Grabham	1	6	7		
Culex (Culex) coronator Dyar and Knab		10			
Culex (Culex) declarator Dyar and Knab	18	9			
Culex (Culex) inflictus and/or *nigripalpus*		12			
***Culex (Culex) inflictus* Theobald**	9	5	3	4	2
***Culex (Culex) nigripalpus* Theobald**	21	37	17	54*	22
***Culex (Culex) quinquefasciatus* Say**	17	12	9	30	12
***Culex (Melanoconion) atratus* Theobald**	5			2	
Culex (Melanoconion) idottus Dyar	16	6			1

Culex (Melanoconion) jocasta Komp and Rozeboom			5		19
Culex (Melanoconion) madininensis Senevet	39	2			
Culex (Micraedes) bisulcatus	44	13			
Deinocerites magnus	9	16	4	5	8
Haemagogus (Haemagogus) splendens Williston			18		7
Limatus durhamii Theobald	13				9
Psorophora (Grabhamia) sp. near *cingulata*		27			3
Toxorhynchites (Lynchiella) guadeloupensis	14				
Trichoprosopon (Isystomyia) perturbans	18	1			3
Wyeomyia (Wyeomyia) greyii Theobald	40	25			
Wyeomyia (Wyeomyia) pertinans			27	7	16

FIGURES

Figure 1. Sampling localities on Barbados island (right), Lesser Antilles (left).

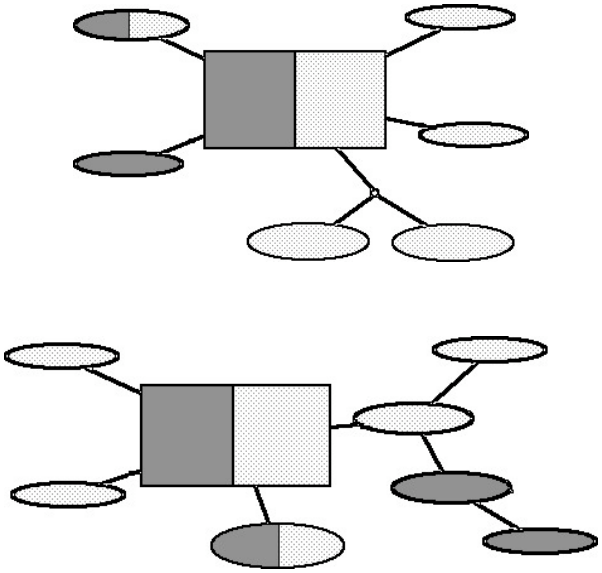

Figure 2. Haplotype networks of northwestern (dotted) and southeastern (grey) *C. flaveola* (top) and *L. barbadensis* (bottom) cytochrome *b*. The rectangles represent the most likely ancestral haplotype (Clement, *et al*., 2000).

CHAPTER 2: THE ROLE OF HOST PHYLOGEOGRAPHY IN STRUCTURING AVIAN MALARIA PARASITE COMMUNITIES

L. Maria E. Svensson and Robert E. Ricklefs

ABSTRACT

We tested whether host phylogeography structures avian malaria parasite communities of passerine birds in the Lesser Antilles. We used the partial Mantel test to determine the significance of correlations between host genetic distance and avian malaria parasite ensemble dissimilarity (difference in the parasite lineages recovered from one particular host species on two islands). The analysis encompassed three species of host on eight islands. We also determine the degree to which the entire parasite community of each island, together with geographic distance, influenced local parasite ensembles. We found no significant correlations between host genetic distance and avian malaria parasite ensemble dissimilarity in any of our three focal species, limited significant correlations between geographic distance and ensemble dissimilarity for two focal species, and highly significant correlation between the entire parasite community and ensemble dissimilarities in one focal species. Most of the ensemble dissimilarities between islands remained unexplained by the Mantel models. We discuss the possibilities of host phylogeographic structure, dispersal capabilities, density, and parasite inter-specific competition in structuring avian malaria parasite communities as well as the possibility that these communities are stochastic.

INTRODUCTION

Elucidating the mechanisms that structure biological communities is one of the central goals in ecology (*e.g*. Ricklefs and Schluter, 1993; Weiher and Keddy, 1999). Most often, questions have been applied to free-living organisms, but there is a growing interest in communities of parasitic organisms (Esch *et al*., 1990; Poulin, 2007). One reason for this increasing interest is the unambiguous community boundaries of parasites, often being contained within the body of an individual host or a population of a single host species (Simberloff and Moore, 1997). However, studies on parasite communities on vertebrates have been conducted almost exclusively on metazoan parasites of fish (Poulin, 2007 and references therein). Generalizations concerning parasite communities require consideration of other parasite-host systems.

A parasite community has three levels (Esch *et al*., 1990; Poulin, 2007). At the lowest level is the infracommunity, which is composed of all parasite species infecting a single host individual. At the intermediate level is the component community, which is composed of all parasite species infecting a single host population. The highest level is the compound community, which includes all parasite species infecting all host populations in a particular region. In an island system, for example, host populations are isolated by island; thus, all parasites in all hosts on an island compose the compound community. Within each island, each host species (which on small islands can be considered a single population) harbors a component community of parasites. Finally, within each host species on an island, individual hosts provide the habitats for parasite infracommunities. Although parasite communities should be characterized by all kinds of parasites (helminths, lice, protozoans, etc), one may focus on a particular taxonomic

group. In that case, it is more appropriate to substitute 'component community' with 'ensemble,' which can be defined as a group of organisms sharing both ancestry and resources (Fauth *et al.*, 1996; Magurran, 2004).

Host history is considered an important factor in structuring parasite communities (*e.g.* Ricklefs and Schluter, 1993; Gregory, 1997; Poulin, 2007). Davis and Pedersen (2008), for example, demonstrated that host divergence time (*i.e.*, host phylogeny) is the strongest predictor of protozoan parasite community similarity (*i.e.*, host sharing of parasites) within primates. The importance of host history to parasite community structure is likely to vary depending on how specialized parasites are. That is, in a system where parasites are host specialists, each host species would be expected to harbor a parasite ensemble quite unlike the compound community. In a system where parasites are generalists, however, ensembles should be highly contingent upon the compound community. Host history may be particularly important in island systems, because parasites most likely colonize islands through their vertebrate hosts, in which they are more prevalent, than within their dipteran vectors, in which they are less prevalent (Lehane, 1990; Staats and Schall, 1996; Gager *et al.*, 2008). For instance, time since arrival of the host species has been deemed an important predictor for how component communities of freshwater fish parasites are structured (Guégan and Kennedy, 1993; reviewed in Poulin, 2007). Thus, if host history were important in predicting parasite communities on islands, we would expect parasite ensemble dissimilarity (which is equivalent to beta diversity; Magurran, 2004) between islands to be positively correlated with host genetic distance.

In this study, we consider pigmented haemosporidian (malaria) parasites of birds. Currently, more than 200 morphological species of avian malaria parasites (*Plasmodium* and *Haemoproteus*) have been described (Valkiunas, 2005), but studies on the genetic variation in parasites have uncovered many times that number of distinct lineages, which implies substantial overlooked cryptic diversity (Bensch *et al*., 2004). Although strict host specialization, that is, specialization of one parasite lineage on one host species, appears to be rare in this group of parasites (Fallon *et al*., 2005), common parasite lineages are often found more frequently on one host species than on others (Ricklefs *et al*., 2004). Single avian malaria parasite lineages can be widespread and abundant (*e.g*. Fallon *et al*., 2005; Kimura *et al*., 2006), but parasite prevalence and community composition may vary among regions. In the Lesser Antilles, for example, there is no significant difference in total parasite prevalence (Apanius *et al*., 2000) or in prevalence of the most abundant parasite lineage, *Haemoproteus* sp. haplotype HC (HC; Fallon *et al*., 2003), among islands. However, there is a significant difference in parasite prevalence between host species (some host species are free of parasite infection whereas others, such as *Loxigilla noctis*, exhibit a prevalence greater than 50%; Fallon *et al*., 2003). In addition, both total prevalence and HC prevalence varies significantly within species among islands (the island-times-host species effect; Apanius *et al*., 2000; Fallon *et al*., 2003). The latter authors raised the possibility that host populations evolve independently and that this underlies the geographic structure demonstrated by a significant island-times-host species interaction effect, but no one has tested associations between parasite communities and host phylogeography directly.

Here, we investigate whether host phylogeographic history influences the structure of avian malaria parasite ensembles in three common emberizid passerine (Emberizidae) hosts in the Lesser Antilles. Parasite ensembles might also be highly contingent on the compound community; that is, ensembles on a particular island might merely reflect the relative abundance of parasite lineages represented in all hosts on each island. Therefore, we also investigate whether compound community composition influences avian malaria parasite ensembles. Finally, because movement of parasites between closely situated islands is more likely than movement between islands that are far apart, we also take into account that parasite ensembles that are closer geographically may be more similar. Specifically, we use Mantel tests to resolve the relative contributions of genetic distance and compound community dissimilarity on parasite ensemble dissimilarity while controlling for the effect of geographic distance.

METHODS

Study Site

The present study utilizes avian malaria parasite data from the Lesser Antilles (Figure 1), which sit on the Caribbean-Atlantic plate subduction zone (Wadge, 1994). Islands previously screened for avian malaria parasites are (from north to south) Barbuda, Antigua, Montserrat, Guadeloupe, Dominica, Martinique, St. Lucia, St. Vincent, Barbados, and Grenada (Apanius *et al.*, 2000; Fallon *et al.*, 2003; Fallon *et al*, 2004; Fallon *et al*, 2005). In this study, we include all but Barbuda and St. Vincent, from which samples are limited. Except for Antigua, which is a limestone island, and Barbados, which is a coralline island, the Lesser Antilles are of volcanic origin (Wadge, 1994).

Most islands have been subaerial for approximately 20 million years (Ricklefs and Bermingham, 2007a and references therein). Barbados has been subaerial for less than 1 million years (Speed, 1994). Island populations of birds are generally considered isolated from each other, and distinct phylogeographic breaks occur between many island populations (Ricklefs and Bermingham 2007b), but an assessment of gene flow between the islands in the Lesser Antilles has yet to be attempted.

Sampling of birds and parasites

Bird communities were sampled discontinuously between 1991 and 2007 by mist netting. St. Lucia was sampled both in 1991 and in 2000, and Barbados was sampled both in 1993 and in 2007. The other islands were sampled either in 1991, 1993, or 2002. All sampling took place in the dry season (May to August). DNA was extracted from blood, obtained via sub-brachial venipuncture, and data on the avian malaria parasite lineages recovered from the samples were identified in previous studies by sequencing part of cytochrome *b* (Fallon *et al*., 2005; Chapter 1). Our three focal bird taxa are *Coereba flaveola*, *Loxigilla noctis/barbadensis*, and *Tiaris bicolor*. We chose these species because these are abundant, occur on all Lesser Antillean islands, and exhibit high prevalence of avian malaria parasites. *L. barbadensis* was recently considered a separate species (Buckley and Buckley, 2004), but in this study we group both *L. noctis* and *L. barbadensis*, which will hereafter be referred to as *Loxigilla*. Sample sizes of each species are shown in Table 1.

Parasite lineage diversity

To describe parasite diversity in the Lesser Antilles, we calculated alpha diversities (α: parasite lineage diversity on each island, both in the compound community and within each focal species) and gamma diversity (γ: parasite lineage diversity within the Lesser Antillean region, both in the compound community and within each focal species) using Simpson's index $D = \sum\left(\frac{n_i}{N}\right)^2$, where n_i = the number of individuals in the *i*th lineage and N = the total number of individuals (Magurran, 2004). For the γ diversity analysis, we also included Barbuda and St. Vincent. We used multiple regression to test the effect of sampling effort and number of potential host species present on an island on parasite diversity. The regression was run through a generalized linear model procedure (GLM, software SAS software, version 8.01; SAS Institute Inc., Cary, NC). Potential host species are all species belonging to families from which avian malaria parasites have been recovered in the Lesser Antilles. Thus, we exclude families of birds from which no avian malaria parasites have been recovered (both families that have been screened and considered non-infected and families that have not been sampled at all).

Parasite ensemble dissimilarity

To compare diversities between island pairs we calculated beta diversities, which will henceforth be referred to as "dissimilarities." Because ensemble composition can differ markedly over time in a single locality, with gain or loss of individual lineages sometimes being observed within a decade (Fallon *et al.* 2004), we performed two separate analyses; one included data from the 3-year period 1991-93 only ("1990's") and the second included data from all years (1991-2007; "All-years"). Because Grenada was

sampled only in 2002, and because none of the 4 parasite infections of *T. bicolor* on Grenada was identified, Grenada was excluded from both analyses of *T. bicolor*.

Avian malaria parasite mitochondrial lineage ensemble dissimilarity among islands was calculated in *EstimateS* (Colwell, 2006). When choosing the most appropriate index for representing parasite lineage dissimilarities, we considered indices that best handle small sample sizes and datasets where most lineages are rare, because in our datasets, sample size varies by island and some parasite lineages are much more abundant than others. Therefore, we chose the following three indices that incorporate abundance data when determining similarities between component communities; the abundance-based Chao-Jaccard and Chao-Sørensen indices (Chao, *et al.*, 2005) and the Morisita-Horn quantitative dissimilarity index (reviewed in Magurran, 2004). The Chao-Jaccard and Chao-Sørensen indices are calculated by: $J = \frac{UV}{U + V - UV}$ and $S = \frac{2UV}{U + V}$, where U is the proportion of individuals from one community belonging to shared species and V is the proportion of individuals from the other community belonging to shared species (Chao *et al.*, 2005). Although Chao *et al.* (2005, 2006) demonstrate that the most accurate indices are "estimated" abundance indices, which take into account the probability of missing unseen shared species, Chao *et al.* (2006) cautions against using the estimated indices when there are less than 10 shared species, as is the case in our data. The Morisita-Horn index is calculated as $C_{\mathrm{MH}} = \frac{2\sum(a_i \cdot b_i)}{(d_a + d_b) \cdot (N_a \cdot N_b)}$, where N_a = the total number of individual parasites on island A; N_b = the total number of individual parasites on island B; a_i = the number of individuals of the *i*th lineage on A; b_i = the number of

individuals of the *i*th lineage on B; and d_a and d_b adjusts for abundance of particular parasite lineages through the equation $d_a = \frac{\sum a_i^2}{N_i^2}$ (Magurran, 2004). This particular index has demonstrated the least sensitivity to both sample size and diversity (Wolda, 1981). In addition, it performs extremely well (*i.e.*, it accurately estimates dissimilarities) when α diversity is low, as is the case in our system (Wolda, 1981). All three indices range from 0, indicating no sharing of species between communities, and 1, indicating community identity. Because it is more intuitive (Magurran, 2004), we subtracted the index from one, creating dissimilarity indices as opposed to similarity indices. Now, 0 indicates high similarity and 1 indicates high dissimilarity.

Compound community dissimilarity

Two datasets, all-years and 1990's, were produced for compound community dissimilarity for each focal species. For each matrix, all parasite lineages recovered from all host species on an island, minus the focal species data, were included. For example, when performing the analyses on parasite ensembles of *C. flaveola*, the compound community matrix did not include any parasite data for *C. flaveola* individuals on a particular island. Had we not excluded the focal species data, our results would be biased in favor of a positive correlation between the ensemble and the compound community structure, especially in datasets where several individuals from the focal species were infected. The same indices that were computed for ensembles were also computed for the compound communities.

Host genetic distance

Cytochrome *b* was amplified using primers H16065 (Helm-Bychowski and Cracraft, 1993) and L14990 (Kocher, 1989 in Helm-Bychowski and Cracraft, 1993) and sequenced in both the forward and reverse directions on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). A minimum of 2 individuals from each of the 3 host species and each island were successfully sequenced, except for in *T. bicolor* on Antigua of which we successfully sequenced only one individual. From some islands, *e.g.* Barbados and St. Lucia, we sequenced as many as 5 individuals. Sequences were edited and aligned in SeqMan™ II 4.0 (DNASTAR Inc., Madison, WI). Host phylogeographic variation was then estimated from pairwise distances that were corrected for ancestral polymorphism in Arlequin 3.11 (Excoffier *et al.*, 2005) by the equation $D_C = P_iXY - \frac{P_iX + P_iY}{2}$, where P_i is the average pairwise nucleotide difference between two populations, P_iXY is the average pairwise nucleotide difference between two populations from two different islands, and P_iX and P_iY are the average within-population pairwise nucleotide differences. Correcting for ancestral polymorphism is important in studies addressing questions involving population divergence instead of gene divergence (Edwards and Beerli, 2000). Effectively, this procedure measures inter-island population divergence controlling for how much of the variation is caused by intra-island polymorphisms. We used 897 bp and 29 sequences of *C. flaveola*, 941 bp and 38 sequences of *Loxigilla*, and 827 bp and 25 sequences of *T. bicolor*. We also created a haplotype network of each species in TCS v.1.21 (Clement *et al.*, 2000). The TCS program produces a statistical parsimony network using methods described by Templeton

et al., (1992; Clement *et al*., 2000). Ambiguities were solved based on most likely connections described in Posada and Crandall (2001).

Mantel tests

Pairwise geographic distances between islands were obtained from a wall map published by the Oxford Cartographers, U.K. Partial Mantel tests (Smouse *et al*., 1986; reviewed in Legendre and Legendre, 2000), in which we estimated the correlation between ensemble dissimilarity and the two independent variables (host genetic distance and compound community dissimilarity) while controlling for the effect of geographic distance, were performed in FSTAT 2.9.3 (Goudet, 2002) on both datasets (all-years and 1990's). We performed 10,000 permutations to create a null sampling distribution of the normalized Mantel statistic, *r* (Legendre and Legendre, 2000). In addition to the partial Mantel test, we performed simple Mantel tests to calculate the percent variation attributed to compound community dissimilarity and geographic distance separately, following the procedures of Telles and Diniz-Filho (2005). Simple Mantel tests were performed only in those cases where the partial Mantel test produced significant correlations of both independent variables.

RESULTS

Community diversity

The reciprocal Simpson's diversity indices ($1/D$) are listed in Table 2. This reciprocal index ranges from 1 (low diversity) to *N* (high diversity), where *N* is the total number of parasite lineages. The total diversity, or γ diversity, of avian malaria parasites in the

Lesser Antilles has an index value of 5.7 (Table 2). St. Lucia supported the greatest compound diversity ($\alpha = 4.4$). Throughout the Lesser Antilles, *Loxigilla* supported the lowest avian malaria parasite diversity ($\gamma = 2.3$) and *C. flaveola* supported the greatest diversity ($\gamma = 3.9$), however this is not reflected in α diversity indices, where *T. bicolor* most often supported the lowest diversity and *Loxigilla* supported the lowest diversity only on St. Lucia. One abundant parasite lineage, HD, was found almost exclusively on *C. flaveola* (Fallon *et al.*, 2004); this one lineage is probably largely responsible for the higher diversity in *C. flaveola*. *C. flaveola* also shared parasite lineages with *Loxigilla* and *T. bicolor*. The number of sequenced parasite infections was not related to parasite diversity (p=0.72) whereas number of potential host species was significantly related (F=24.0, p=0.001, R^2=0.75) to parasite diversity exhibited on an island (slope of the regression, b=0.141 ± 0.029 SE, t=4.90, p=0.001; Figure 2).

Community dissimilarities

Avian malaria parasite ensemble dissimilarity among islands varied between pairwise comparisons from 0 (complete similarity) to 1 (complete dissimilarity; *i.e.*, no shared lineages). In *C. flaveola*, there were few instances of both complete similarity (1 of 28 comparisons) and complete dissimilarity (3 of 28). In *Loxigilla*, there were no instances of complete similarity in parasite lineage ensembles but 9 instances of complete dissimilarity. In *T. bicolor*, complete dissimilarity between parasite lineage ensembles dominated (13 of 21 comparisons). Both ensembles and compound communities were on average more dissimilar than similar (Table 3, Figure 3). None of the compound communities were identical.

Host genetic distance

Loxigilla sp. exhibited the greatest genetic divergence between islands, and *T. bicolor* the least (Table 3, Figure 4). The values are slightly different in the two datasets because Grenada was excluded in all 1990's analyses. Barbados exhibited the highest divergence from the other islands in all species. The maximum corrected genetic distances were found between Barbados and Grenada for *C. flaveola* (0.66%), Barbados and Montserrat/Guadeloupe for *Loxigilla* (0.92%), and Barbados and the four islands Antigua, Montserrat, Dominica, and Martinique in *T. bicolor* (0.04%). Most pairwise comparisons within *T. bicolor* show no genetic divergence. There is a large phylogeographic disjunction within *Loxigilla* between the southern islands of Martinique, St. Lucia, Barbados, and Grenada and the northern islands of Dominica, Guadeloupe, Montserrat, and Antigua (Figure 4). Within these two clusters, the average genetic distance is 0.01% (±0.02%) and 0.23% (±0.12%) for the North and South, respectively. The average genetic distance separating the northern and southern clusters is 0.70% (±0.17%). Equivalent disjunctions are not found in either *T. bicolor* or *C. flaveola*, although Grenada (0.31%, ±0.15% different) and Barbados (0.43%, ±0.10% different) are more distinct from the other islands phylogeographically in *C. flaveola* (Figure 4).

Mantel tests

Figure 5 shows how parasite ensemble dissimilarity correlates with genetic distance, geographic distance, and compound community dissimilarity. In some cases, genetic distance is strongly correlated with geographic distance ($r > 0.6$), and in one case, compound community dissimilarity is strongly correlated with geographic distance (r >

0.6; Table 4). The three indices (Chao-Jaccard, Chao-Sørensen, and Morisita-Horn) produced slightly different values but were largely in agreement on the final results (Table 5). The correlation between genetic distance and ensemble dissimilarity and between compound community dissimilarity and ensemble dissimilarity (Figure 5) were calculated separately and will hereafter be referred to as the genetic model and the compound community model, respectively. Because we use the same datasets to test two hypotheses (the correlation with genetic distance and the correlation with compound community dissimilarity), we use the Bonferroni correction and consider significance at a p-value lower than 0.025.

Three models were significant, as determined by significant R^2 values (Table 5): one genetic model (*Loxigilla*, all-years dataset) and two compound community models (*T. bicolor*, both datasets). Significant correlation coefficients usually accompanied significant models. The genetic model for *Loxigilla* explained approximately 30% of the variation. However, this could not be attributed to genetic distance, but solely to geographic distance (Table 5). The compound community model for *T. bicolor* explained more than 50%, and in one case 69%, of the variation (Table 5). This variation is attributed primarily to compound community dissimilarity, but geographic distance alone explained up to 19% of the variation (Table 6).

DISCUSSION

Five results emerged from our analyses. First, α diversity is significantly related to number of potential host species. Second, host genetic distance does not correlate with parasite ensemble dissimilarity and is, therefore, unlikely to be a determinant of ensemble

composition. Third, some ensembles change in parallel with the compound communities indicating that the host contracts whatever parasite lineages are present on an island. Fourth, some of the variation in ensemble structure can be explained by geographic distance. Fifth, most of the variation in ensemble structure is unrelated to host genetic distance, compound community structure, and geographic distance.

α diversity

Parasite diversity increases with an increasing number of potential host species, suggesting that diverse host communities have the potential to support more parasite lineages (Figure 3). Because parasite specialization is rare in the Lesser Antilles (Fallon *et al*., 2005), any particular host species is likely to support a more diverse parasite ensemble if they themselves are members of a more diverse community.

Host genetic distance and ensemble dissimilarity

The variation in genetic diversity between island populations in the three focal host species is intriguing. The Lesser Antillean endemic *Loxigilla* has diversified extensively in the Lesser Antilles (Table 3, Figure 5, Appendix 1), even within *L. noctis*. *C. flaveola* also exhibits relatively high diversity in the Lesser Antilles, but the northern and central islands are not phylogeographically distinct (Figure 5, Appendix 1). *T. bicolor*, in contrast, is relatively uniform (Table 3, Figure 5, Appendix 1). Thus, if the phylogeographic history of hosts causes parasite ensembles to differ between islands, one would expect *Loxigilla*'s parasite ensembles to vary greatly between islands, whereas *T. bicolor*'s parasite ensembles would be uniform, mirroring the different levels of host

genetic divergence. On the contrary, *T. bicolor*'s ensembles exhibit the greatest average dissimilarity, whereas *Loxigilla*'s ensembles exhibit the greatest average similarity (Table 3). Within *C. flaveola*, little genetic divergence is observed between most islands (average nucleotide difference = 0.01% when Grenada and Barbados are removed), whereas Grenada and Barbados are phylogeographically distant from the other islands (average nucleotide difference = 0.31% and 0.43%, respectively) and highly divergent from each other (average nucleotide difference is= 0.66%). The Grenada and Barbados isolation is reflected in the statistical parsimony haplotype network (Figure 4). Pairwise genetic distances are presented in Appendix 1. The average ensemble dissimilarity between Grenada and all other islands (Chao-Jaccard index, $J_{GD/others} = 0.79, \pm 0.25$) and between Grenada and Barbados ($J_{BA/GD} = 0.88$) are higher than the average ($J_{avg} = 0.66, \pm 0.27$), which is what we predict if host genetic distance correlates with parasite ensemble dissimilarity. However, the average ensemble dissimilarity between Barbados and all other islands ($J_{BA/others} = 0.57, \pm 0.30$) is lower than the average, which is contrary to what we predict if host genetic distance correlates with parasite ensemble dissimilarity. Thus, within our datasets, we have contrasting patterns of ensemble dissimilarity and host genetic distance.

Consequently, the partial Mantel model of genetic distance and ensemble dissimilarity performed poorly for most comparisons (Table 5), and where the model is significant (in *Loxigilla* only) it is entirely due to a significant correlation between geographic distance and ensemble dissimilarity (Table 5). We also found that genetic and geographic distances are strongly correlated (Table 4). However, the simple Mantel tests ascribe all the variation in ensemble composition to geographic distance (Table 5),

which means that genetic distance alone, even when we do not control for the effect of geographic distance, does not explain any of the ensemble dissimilarity. Taken together, our results lead to the rejection of the hypothesis that host genetic distance underlies ensemble dissimilarity.

Compound community diversity

Avian malaria parasites are to a certain extent generalists, although some are restricted to a single host species (Fallon *et al.*, 2005; Ricklefs *et al.*, 2005). Hence, with few limitations on the parasites' capability of exploiting different host species, one would expect ensembles to closely mirror the compound community, especially when the phylogeographic histories of the hosts have no influence in structuring parasite ensembles. Fallon *et al.* (2003) hinted at the discrepancy between compound communities and ensembles when they demonstrated a significant island-times-host effect of HC prevalence in the absence of a significant island effect (in which they considered the compound community). Here we extended the analysis to investigate the variation between islands in all lineages so far recovered. We show that despite generalist behavior, avian malaria parasite ensembles in the Lesser Antilles do not always mirror the compound community, except for in the case of the *T. bicolor* ensembles (Tables 5 and 6). Considering that Barbados may bias the correlation, since one lineage dominates the compound community there and the prevalence of this lineage is extremely high in *T. bicolor*, we removed Barbados from the matrices and re-analyzed the remaining data. This did not change the significance or correlations between ensemble dissimilarity and compound community dissimilarity (results not shown).

Host genetics, parasite compound communities, and parasite ensembles

The high genetic divergence (Table 3, Appendix 1) between islands suggest that the *Loxigilla* island populations have been isolated from each other for a long time. The low genetic divergence within *T. bicolor*, on the other hand, suggests that the *T. bicolor* island populations are not phylogeographically structured. We do not attempt to distinguish between time of colonization and levels of gene flow within *T. bicolor*; either of these mechanisms would produce relatively similar phylogeographic patterns. *C. flaveola*, on the other hand, exhibits no clear, consistent phylogeographic pattern within the Lesser Antilles, except that St. Lucia and Grenada seem more phylogeographically distant from the other islands than average. That is, the corrected pairwise genetic distances are generally low, but there also seems to be high diversity within islands (Appendix 1), possibly caused by multiple colonization events or large effective population sizes. The Grenada separation is consistent with results in Seutin *et al.* (1994), where a clear phylogeographic disjunction separates Grenada/St. Vincent from the northern islands.

Combining the results from our two models, we see an intriguing pattern: the host populations exhibiting no phylogeographic structure (those of *T. bicolor*) reflect the community composition of the compound community of which they are members, whereas phylogeographically structured host populations (those of *Loxigilla*) do not mirror the compound community at all. Ensembles of both unstructured and structured populations correlated to a similar low degree with geographic distance. As mentioned before, because ensemble dissimilarity and host genetic distance in *Loxigilla* are not correlated, host phylogeographic history is unlikely to be the primary underlying force in

structuring parasite communities. Nevertheless, that the compound community is not mirrored in *Loxigilla*'s parasite ensembles argue for this host population having evolved long enough with the parasite community for independent structuring among islands of the ensembles to occur. That is, parasite ensembles in *Loxigilla* are not stochastic, but are likely the result of local coevolution between hosts and parasites.

In contrast, the unstructured host populations of *T. bicolor* exhibit a high positive correlation between compound community dissimilarity and ensemble dissimilarity. This high correlation is not the result of regional homogeneity of parasite communities; pairs of islands are in general more dissimilar than similar, both in their parasite compound communities and ensembles (Table 3, Appendix 2). Thus, *T. bicolor* populations closely mirror the compound community structure suggesting that their defenses against parasites do not discriminate among parasite lineages.

The unexplained variation

For vector-borne parasites, several factors are likely to be important in structuring communities: for example, dispersal capability of hosts may homogenize relatively isolated communities (Poulin, 2007), inter-specific competition may exclude certain parasite species from either particular ensembles or compound communities (*e.g.* Richie, 1988; de Roode *et al.* 2005), and abundance and/or densities of hosts may influence the survival of parasite populations (Price, 1990). These "hosts" could be either the birds or the dipteran vectors in the case of malaria parasites.

Frequent movement of parasites between islands may reduce the likelihood of detecting correlations between host phylogeography and ensemble dissimilarity.

However, the great average dissimilarity of compound communities between islands suggest that if movement of parasites is frequent, it has not succeeded in homogenizing the parasite communities.

Interspecific competition could play a role in structuring parasite communities either through direct competition for resources or apparent competition resulting from strain-transcending immunity (Richie, 1988; de Roode *et al.*, 2005). Avian malaria parasites share one common resource—haemoglobin—and competition for this resource has been shown to exist in other vertebrate malaria parasite hosts (de Roode *et al.*, 2005). Yet, on average only 28% of birds exhibit infections in their peripheral blood tissue in the Lesser Antilles (Fallon *et al.*, 2005), and parasitemia are generally low (Apanius *et al.*, 2000); hence, there are plenty of non-utilized resources present. The fact that few birds exhibited multiple infections (Durrant *et al.*, 2008; Fallon *et al.*, 2005) could be the result of competitive exclusion (Bensch *et al.*, 2007), or just a low probability of detection. Alternatively, the rarity of multiple infections could be the result of a low probability that two infected vectors, carrying two different parasite lineages, bite the same individual bird. The low prevalence of avian malaria parasites in Lesser Antillean birds coupled with the extremely low prevalence in vectors observed in another Neotropical area (Gager *et al.*, 2008) argues strongly for the latter explanation; *i.e.*, parasites are unlikely to be given the opportunity to competitively exclude each other in nature. Thus, although parasite competition has been observed in infracommunities (de Roode *et al.*, 2005), the role of competition in structuring component communities is likely minimal. Nevertheless, it is intriguing that the most abundant lineage in the Lesser Antilles, HC, is absent or in low prevalence in compound communities where its sister lineage, HH,

predominates (Fallon *et al*., 2005), and that the prevalence of HC alternates between hosts on different islands (Fallon *et al*., 2003), indicating some level of interaction even at the component community level.

Host and vector abundance must have an impact on parasite communities. Although we have no quantitative data on the abundance of our focal species, it is clear that they are currently some of the most abundant birds in the Lesser Antilles, and it is highly unlikely that their populations would not be able to maintain a parasite population, at least on an ecological time-scale. On an evolutionary time-scale, abundance of individuals within host populations may have fluctuated and exhibited what is referred to as taxon cycles (Wilson 1959,1961); that is, recurrent extinction and re-colonization of island populations (Ricklefs and Cox, 1972; Ricklefs and Bermingham, 1999). Taxon cycles of host populations would likely result in frequent local extinctions of specialized parasite lineages. Local extinctions of parasite lineages in the Lesser Antilles are thought to be frequent (Fallon *et al*., 2005). For example, local extinctions likely caused the current disjunct distribution of parasite lineage HD, which was recovered most frequently from *C. flaveola*, but exhibited some spillover to other species (Fallon *et al*., 2005). Thus, some patterns in the Lesser Antillean avian malaria system are consistent with what one would predict if taxon cycles occurred. Testing the relationship between host taxon cycles and parasite community structure has yet to be attempted. Unfortunately, we know nothing of vector abundance or communities in the Lesser Antilles, which could influence parasite communities in the same way as host communities could.

Finally, it is possible that parasite ensembles and compound communities in the Lesser Antilles are assembled stochastically. This is especially likely when parasites are

generalists, and has been considered the most likely explanation in freshwater eel helminth community assemblages (Kennedy, 1990). Stochasticity is supported by the high dissimilarity both between compound communities and ensembles, of which little is explained by geographic distance. However, the inconsistency between compound community dissimilarity and ensemble dissimilarity in two of our three focal species suggests that some demographic variable(s) within each host species influences parasite community structure. In addition, some host species, although extensively sampled, do not harbor a parasite lineage that is a common part of the host's ensemble in most of its range, even when it is present in the compound community. For example, 176 *C. flaveola* were sampled on Grenada. Of these, 41 were infected with avian malaria parasites and 32 of these infections were identified. None of these infections were identified as HC, which is the most frequently detected lineage in *C. flaveola* on the other islands. This is in spite of HC being present in other hosts on Grenada. This observation suggests that an infection obstacle, whether host resistance, competition, or vector dynamics, has shaped Grenada's *C. flaveola* ensemble. Many factors of this kind, expressed uniquely in different host populations, would give the appearance of randomness in parasite ensembles.

Any tests of correlations between ensemble dissimilarity and alternative, or rather, additional structural factors such as parasite competition and/or host abundance, will likely fail to take into account that hosts are not passive exploitable habitats. Hosts and malaria parasites represent a true interaction, where parasites incur costs to the hosts (Marzal *et al.*, 2005; Gilman *et al*, 2007) and hosts evolve defenses against the parasites (van Riper *et al.* 1986). Thus, the influence of host history, as determined in this study

by phylogeography, on parasite ensembles may be masked by additional forces structuring communities, but host history most certainly influences community structure of avian malaria parasites to some degree.

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TABLES

Table 1. Sample sizes (sequenced infections/total number sampled) for the two different datasets (all-years and 1990's).

	AN	MO	GU	DO	MA	SL	BA	GD
All-years								
Compound	21/94	63/141	53/167	58/222	32/139	118/41	78/342	51/530
C. flaveola	4/16	6/18	21/28	11/18	3/7	24/61	19/66	32/176
Loxigilla	11/20	6/11	6/29	15/30	16/26	46/71	37/93	1/40
T. bicolor	1/18	2/10	8/11	7/10	3/7	9/18	20/24	0/30
1990's								
Compound	21/94	63/141	53/167	58/222	32/139	90/213	19/85	N/A
C. flaveola	4/16	6/18	21/28	11/18	3/7	10/28	3/10	N/A
Loxigilla	11/20	6/11	6/29	15/30	16/26	23/35	11/21	N/A
T. bicolor	1/18	2/10	8/11	7/10	3/7	4/9	3/7	N/A

Table 2. Alpha (α) and gamma (γ) diversity of avian malaria parasite mitochondrial lineages in the Lesser Antilles. The first row represents the diversity in the compound community, and the final three rows represent the diversity in the three focal host species. The minimum diversity is 1 and the maximum is the number of lineages present in each community. AN=Antigua, MO=Montserrat, GU=Guadeloupe, DO=Dominica, MA=Martinique, SL=St. Lucia, BA=Barbados, GD=Grenada.

	α diversity								Mean α	γ	$\frac{\gamma}{\alpha}$
	AN	MO	GU	DO	MA	SL	BA	GD			
Compound	2.5	2.7	4.4	3.9	3.5	4.4	1.1	3.8	3.3	5.7	1.7
C. flaveola	1.0	3.0	2.3	1.4	3.0	2.7	1.3	1.6	2.0	3.9	2.0
Loxigilla	1.2	2.0	2.6	2.5	1.5	1.7	1.0	1.0	1.7	2.3	1.4
T. bicolor	1.0	2.0	1.3	1.8	1.0	2.3	1.0	N/A	1.5	2.9	1.9

Table 3. Mean ensemble dissimilarity, mean compound community (CC) dissimilarity, average corrected genetic distance (percent nucleotide difference), and standard deviations for each focal species divided in two different sampling periods. Because all similarity indices used were comparable, only results from the Chao-Jaccard index are shown here.

	Mean ensemble dissimilarity	SD	Mean CC dissimilarity	SD	Mean genetic divergence	SD
All-years						
C. flaveola	0.662	0.275	0.665	0.265	0.167	0.192
Loxigilla	0.674	0.307	0.615	0.218	0.450	0.329
T. bicolor	0.799	0.296	0.504	0.216	0.009	0.015
1990's						
C. flaveola	0.633	0.301	0.634	0.268	0.118	0.181
Loxigilla	0.577	0.258	0.628	0.227	0.447	0.364
T. bicolor	0.792	0.380	0.518	0.218	0.009	0.015

Table 4. Correlations between geographic distance and compound community dissimilarity (left) and geographic distance and genetic distance (right). All three ensemble dissimilarity indices produced comparable values and only the Chao-Jaccard index results are shown. Where only 7 islands are included, Grenada is the excluded island. One asterisk denotes an associated p-value smaller than 0.05 and two asterisks denotes a p-value smaller than 0.025.

	No. islands	$r_{CC/Geo}$	$R^2_{CC/Geo}$	$r_{Gen/Geo}$	$R^2_{Gen/Geo}$
All-years					
C. flaveola	8	0.61**	0.37**	0.52**	0.28**
Loxigilla	8	0.29	0.08	0.68**	0.47**
T. bicolor	7	0.38	0.14	0.62*	0.38**
1990's					
C. flaveola	7	0.47*	0.22*	0.60**	0.36**
Loxigilla	7	0.38	0.14	0.76**	0.58**
T. bicolor	7	0.38	0.14	0.62*	0.38**

Table 5. Partial Mantel test results with all samples included and from the 1990's only; $R^2_{ED/Gen}$, variation described by the ensemble dissimilarity/genetic distance model; $r_{ED/Gen}$, partial correlation coefficient of ensemble dissimilarity and genetic distance controlling for the effect of geography; $R^2_{ED/CC}$, variation described by the ensemble dissimilarity/compound community dissimilarity model; $r_{ED/CC}$, partial correlation coefficient of ensemble dissimilarity and compound community composition; $r_{ED/Geo}$, correlation coefficient of geographic distance and ensemble dissimilarity. One asterisk denotes a p-value smaller than 0.05, two asterisks denotes a p-value smaller than 0.025.

	$R^2_{ED/Gen}$	$r_{ED/Gen}$	$R^2_{ED/CC}$	$r_{ED/CC}$	$r_{ED/Geo}$
All-years					
C. flaveola					
Chao-Jaccard	0.02	-0.03	<0.01	-0.04	0.09
Chao-Sørensen	0.03	-0.02	0.02	0.09	0.14
Morisita-Horn	0.02	-0.02	<0.01	0.06	0.09
Loxigilla					
Chao-Jaccard	0.31**	-0.06	0.15	0.18	0.36
Chao-Sørensen	0.37**	-0.05	0.17	0.14	0.41*
Morisita-Horn	0.31**	-0.01	0.16	0.15	0.40*
T. bicolor					
Chao-Jaccard	0.26	0.32	0.53**	0.67**	0.51**
Chao-Sørensen	0.28	0.32	0.53**	0.66**	0.53**
Morisita-Horn	0.27	0.33	0.69**	0.79**	0.52**

1990's					
C. flaveola					
Chao-Jaccard	0.06	-0.22	0.06	-0.21	-0.20
Chao-Sørensen	0.03	-0.16	0.03	-0.17	-0.13
Morisita-Horn	0.05	-0.23	0.02	-0.07	-0.14
Loxigilla					
Chao-Jaccard	0.09	-0.12	0.05	0.23	0.09
Chao-Sørensen	0.13	-0.11	0.04	0.19	0.14
Morisita-Horn	0.10	-0.06	0.03	-0.02	0.16
T. bicolor					
Chao-Jaccard	0.26	0.40	0.50**	0.66**	0.50**
Chao-Sørensen	0.27	0.41	0.49**	0.64**	0.51**
Morisita-Horn	0.26	0.40	0.63**	0.74**	0.50**

Table 6. The variation explained by the partial compound community model from Table 5 is partitioned into percent explained by compound community dissimilarity and geographic distance combined (Both), only compound community dissimilarity (CC), and only geographic distance (Geo). Only models producing significant correlations for both independent variables are shown here. All data below is from *T. bicolor* models. The variable contributing the most to the model is boldfaced.

	Both	CC	Geo
All-years			
Chao-Jaccard	35	**51**	14
Chao-Sørensen	33	**48**	19
Morisita-Horn	29	**61**	10
1990's			
Chao-Jaccard	35	**51**	14
Chao-Sørensen	36	**48**	16
Morisita-Horn	27	**60**	12

FIGURES

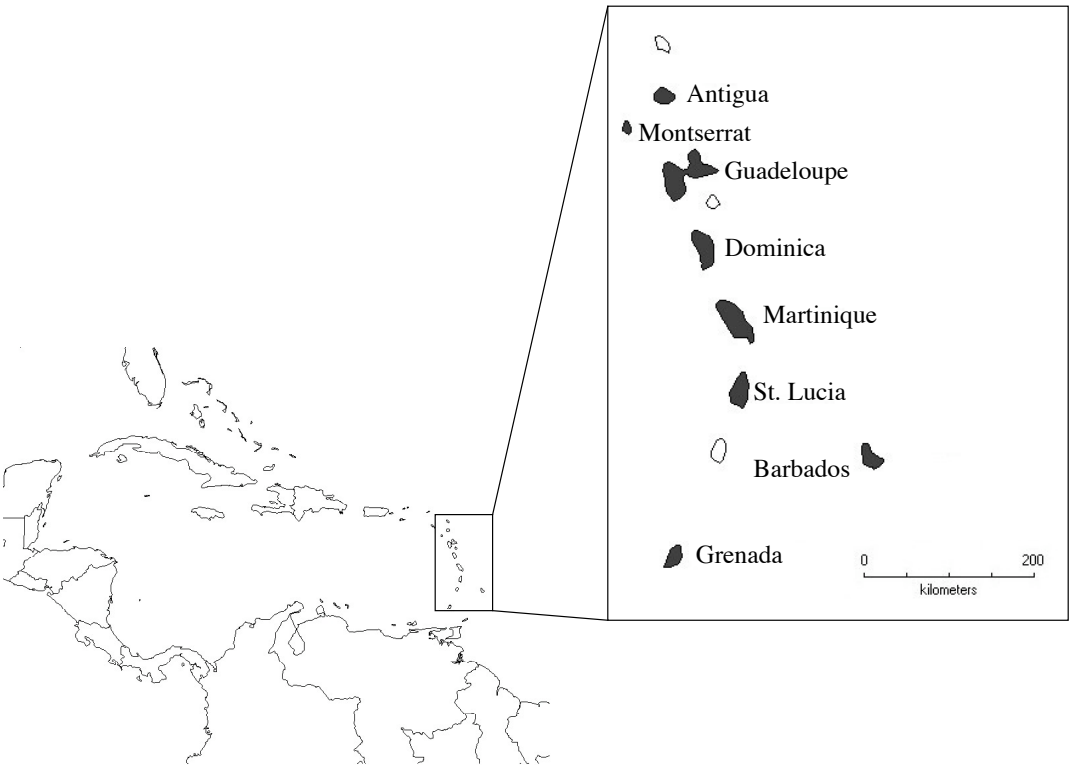

Figure 1. The West Indies (left) and part of the Lesser Antilles (right). Islands included in this study are labeled and shown in black.

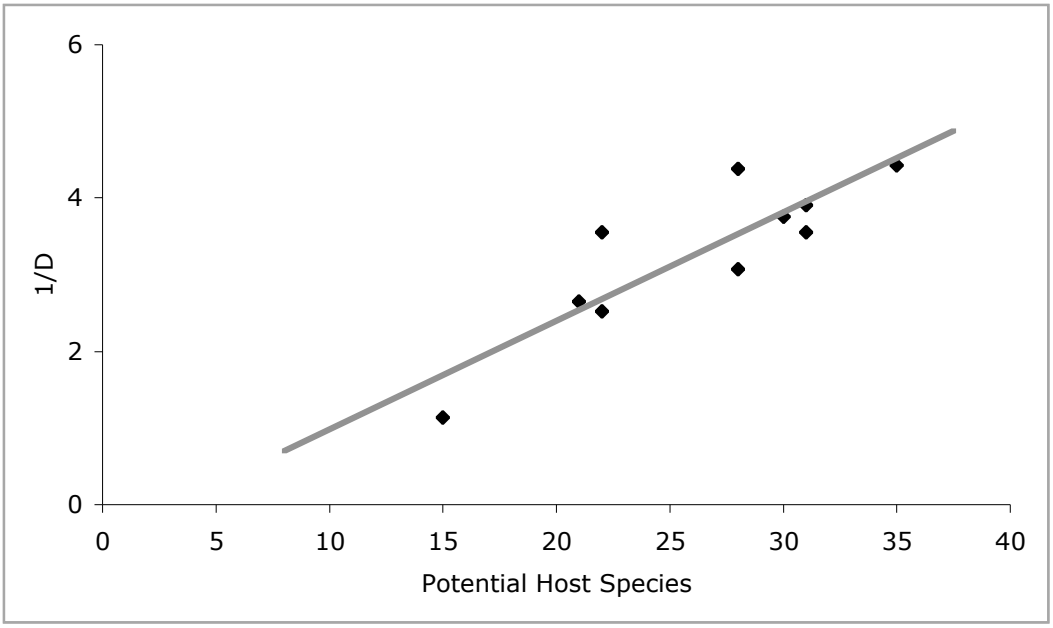

Figure 2. The relationship between the number of potential host species present in a community (x-axis) and the reciprocal Simpson's index of parasite lineage richness (y-axis). The line equation is $y = 0.1414x - 0.4233$. The *y*-intercept (-0.4233) is not significantly different from zero (p=0.60).

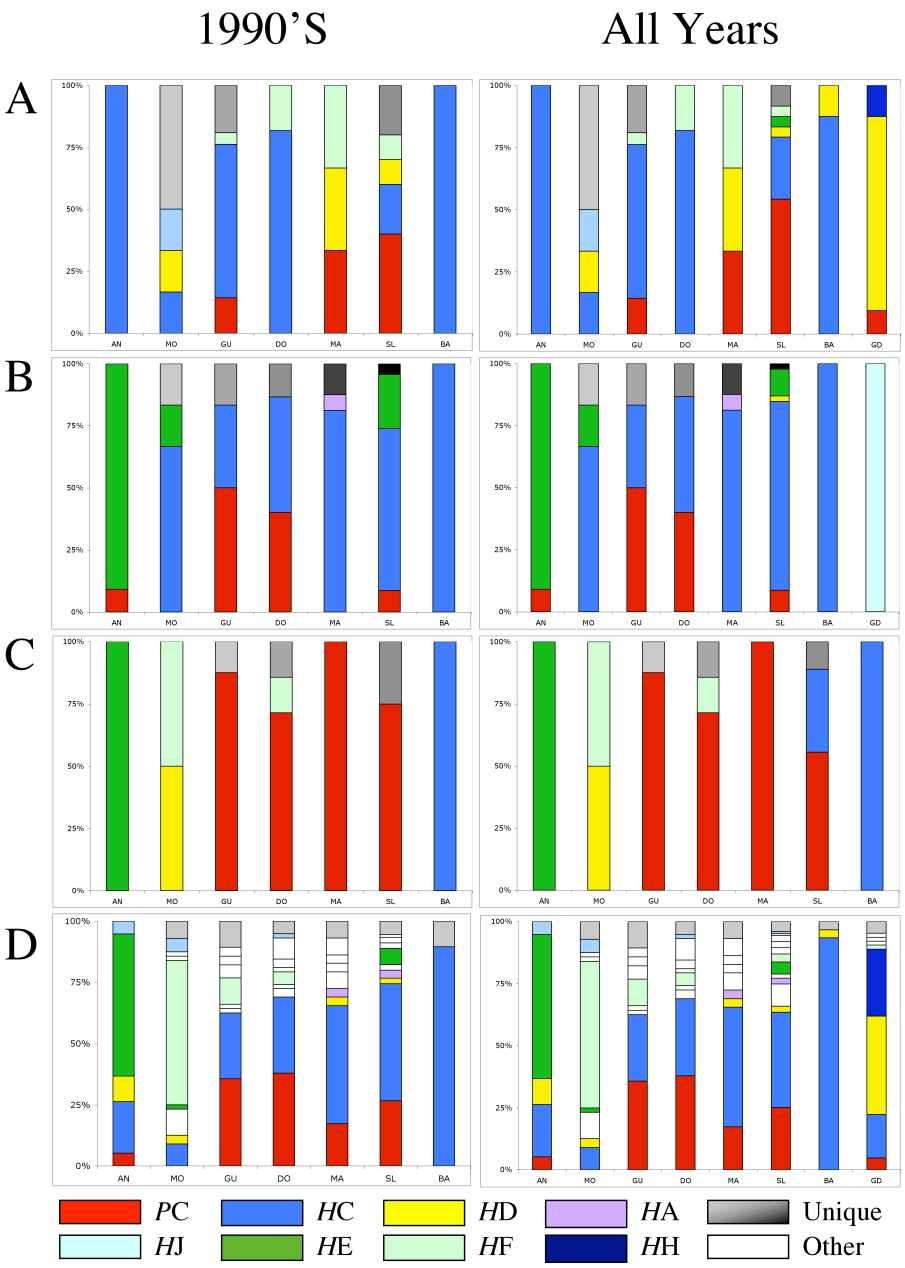

Figure 3. Avian malaria parasite ensembles in *C. flaveola* (A), *Loxigilla* (B), *T. bicolor* (C), and the compound community (D) in the early 1990's and over all years sampled. *Haemoproteus* sp. lineages start with an "*H*" and *Plasmodium* sp. lineages start with a "*P*." Grey areas represent haplotypes unique to a particular island. Parasite lineages recovered from hosts other than *C. flaveola*, *Loxigilla*, and *T. bicolor* are shown in white. Antigua (AN), Montserrat (MO), Guadeloupe (GU), Dominica (DO), Martinique and

(MA) were only sampled in the early 1990's; thus the ensembles on these islands are identical in the graphs both on the left and on the right. St. Lucia (SL) and Barbados (BA) were sampled both in the early 1990's and in the 2000's and changed in some cases. Grenada (GD) was sampled only in 2002 and is included only in the graphs to the right.

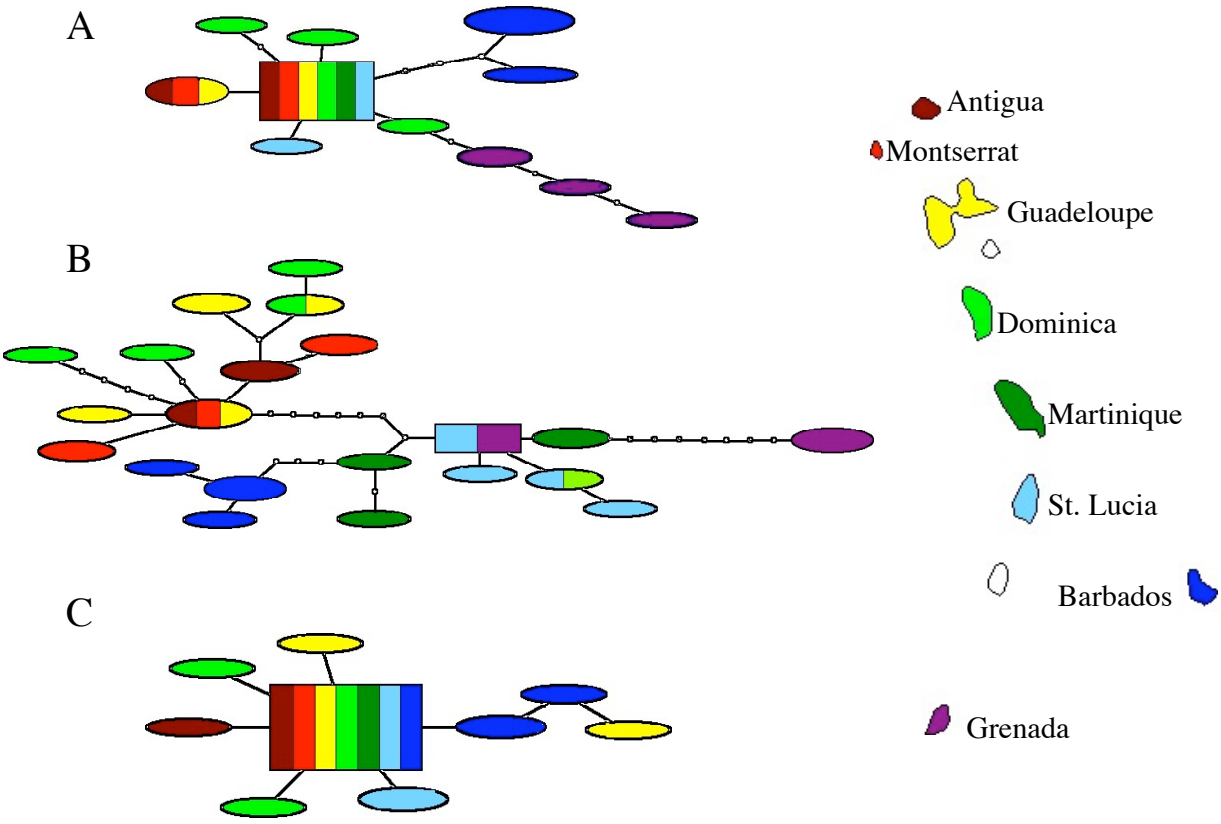

Figure 4. Statistical parsimony haplotype networks of *C. flaveola* (A), *Loxigilla* (B), and *T. bicolor*. Colors correspond to islands (right).

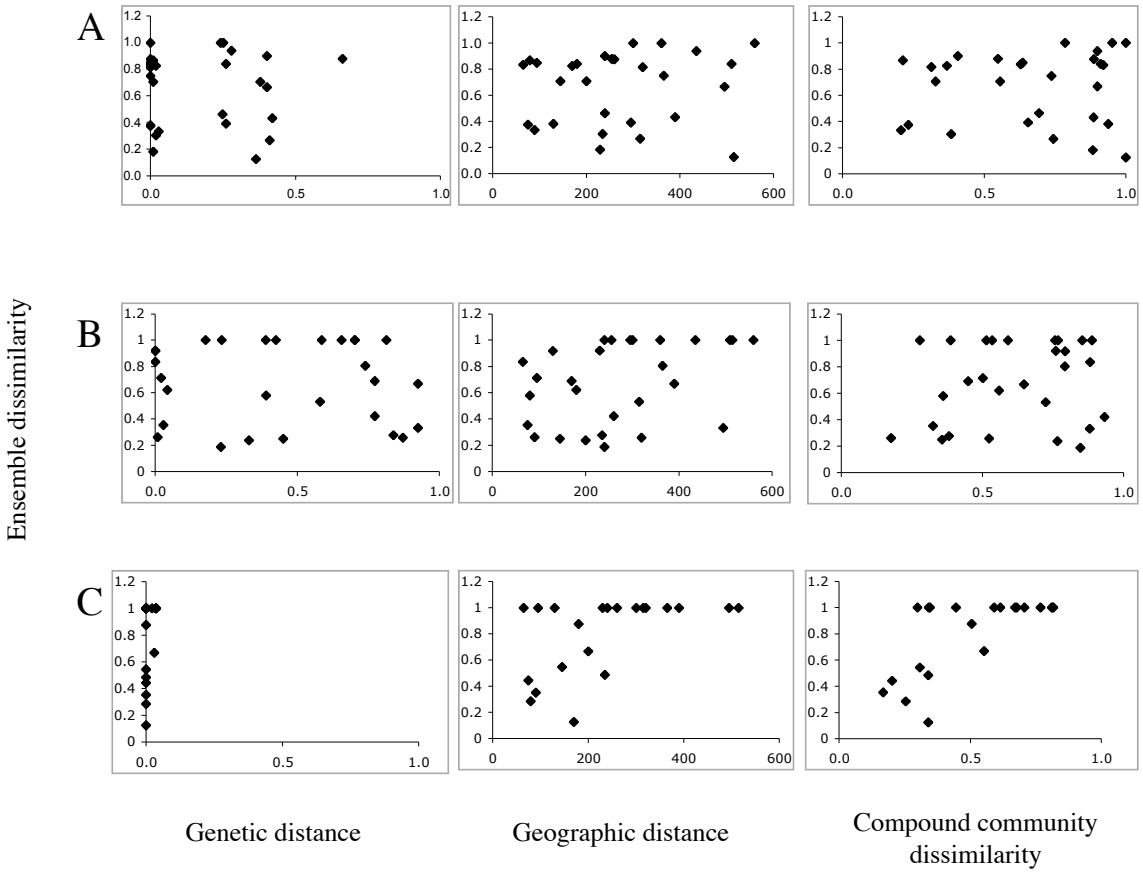

Figure 5. Ensemble dissimilarity (y-axis) and genetic distance, geographic distance (log-transformed), or compound community dissimilarity (x-axes) in *C. flaveola* (A), *Loxigilla* (B), and *T. bicolor* (C).

APPENDICES

Appendix 1. Uncorrected genetic pairwise distance (above diagonal), within-island genetic pairwise distances (diagonal, in bold), and corrected genetic pairwise distances (below diagonal), in percent nucleotide differences.

C. flaveola

	AN	MO	GU	DO	MA	SL	BA	GD
AN	**0.00**	0.04	0.04	0.11	0.00	0.02	0.41	0.38
MO	0.00	**0.11**	0.06	0.17	0.06	0.08	0.50	0.45
GU	0.00	0.00	**0.07**	0.17	0.06	0.08	0.50	0.45
DO	0.01	0.01	0.03	**0.20**	0.11	0.13	0.56	0.47
MA	0.00	0.00	0.02	0.01	**0.00**	0.02	0.45	0.39
SL	0.00	0.00	0.02	0.01	0.00	**0.04**	0.45	0.40
BA	0.36	0.40	0.42	0.41	0.40	0.38	**0.09**	0.84
GD	0.25	0.26	0.28	0.24	0.26	0.25	0.66	**0.26**

Loxigilla

	AN	MO	GU	DO	MA	SL	BA	GD
AN	**0.00**	0.03	0.07	0.30	0.74	0.79	0.83	0.81
MO	0.00	**0.06**	0.13	0.38	0.89	0.96	0.98	0.96
GU	0.00	0.02	**0.15**	0.39	0.94	0.96	1.02	1.00
DO	0.00	0.04	0.01	**0.62**	0.79	0.81	0.91	0.96
MA	0.66	0.77	0.77	0.39	**0.18**	0.17	0.34	0.49
SL	0.74	0.87	0.84	0.45	0.03	**0.11**	0.40	0.51
BA	0.81	0.92	0.92	0.58	0.23	0.33	**0.04**	0.63
GD	0.58	0.70	0.70	0.43	0.18	0.23	0.39	**0.45**

T. bicolor

	AN	MO	GU	DO	MA	SL	BA	GD
AN	**0.00**	0.06	0.05	0.03	0.00	0.00	0.10	0.06
MO	0.00	**0.12**	0.10	0.09	0.06	0.06	0.16	0.12
GU	0.00	0.00	**0.10**	0.08	0.05	0.04	0.13	0.11
DO	0.00	0.00	0.00	**0.06**	0.03	0.03	0.13	0.08
MA	0.00	0.00	0.00	0.00	**0.00**	0.00	0.10	0.06
SL	0.00	0.00	0.00	0.00	0.00	**0.00**	0.09	0.06
BA	0.04	0.04	0.02	0.04	0.04	0.03	**0.12**	0.16
GD	0.00	0.00	0.00	0.00	0.00	0.00	0.04	**0.12**

Appendix 2a. Chao-Jaccard (*J*), Chao-Sørensen (*S*), and Morisita-Horn (*MH*) dissimilarity matrices including data from all years sampled. "ED" subscripts denote ensemble dissimilarities, "CC" subscripts denote compound community dissimilarities minus data of the focal species.

C. flaveola

	Ensemble			Compound Community		
Pairwise comparisons	J_{ED}	S_{ED}	MH_{ED}	J_{CC}	S_{CC}	MH_{CC}
AN-MO	0.83	0.71	0.75	0.92	0.85	0.96
AN-GU	0.38	0.24	0.14	0.94	0.88	0.92
AN-DO	0.18	0.10	0.04	0.88	0.79	0.92
AN-MA	1.00	1.00	1.00	0.95	0.91	0.98
AN-SL	0.75	0.60	0.64	0.74	0.58	0.87
AN-BA	0.13	0.07	0.02	1.00	1.00	1.00
AN-GD	1.00	1.00	1.00	1.00	1.00	1.00
MO-GU	0.85	0.74	0.73	0.64	0.47	0.72
MO-DO	0.84	0.72	0.74	0.63	0.46	0.91
MO-MA	0.88	0.78	0.83	0.89	0.80	0.89
MO-SL	0.82	0.69	0.86	0.31	0.19	0.80
MO-BA	0.67	0.50	0.70	0.90	0.82	0.89
MO-GD	0.84	0.73	0.73	0.91	0.84	0.92
GU-DO	0.33	0.20	0.10	0.21	0.12	0.09
GU-MA	0.83	0.70	0.84	0.37	0.23	0.62
GU-SL	0.30	0.18	0.42	0.38	0.24	0.52
GU-BA	0.43	0.28	0.12	0.89	0.80	0.90
GU-GD	0.94	0.89	0.98	0.90	0.82	0.91
DO-MA	0.87	0.77	0.88	0.21	0.12	0.37
DO-SL	0.71	0.55	0.60	0.33	0.20	0.32
DO-BA	0.27	0.15	0.04	0.75	0.59	0.69
DO-GD	1.00	1.00	1.00	0.79	0.65	0.75
MA-SL	0.38	0.23	0.41	0.23	0.13	0.07
MA-BA	0.90	0.82	0.93	0.41	0.26	0.19
MA-GD	0.39	0.24	0.40	0.66	0.49	0.39
SL-BA	0.71	0.55	0.61	0.56	0.39	0.30
SL-GD	0.46	0.30	0.83	0.69	0.53	0.45
BA-GD	0.88	0.78	0.86	0.55	0.38	0.44

Loxigilla

	Ensemble			Compound Community		
Pairwise comparisons	J_{ED}	S_{ED}	MH_{ED}	J_{CC}	S_{CC}	MH_{CC}
AN-MO	0.84	0.72	0.77	0.88	0.79	0.93
AN-GU	0.92	0.85	0.93	0.79	0.66	0.54
AN-DO	0.92	0.85	0.94	0.76	0.61	0.54
AN-MA	1.00	1.00	1.00	0.85	0.75	0.80
AN-SL	0.80	0.67	0.85	0.79	0.66	0.67
AN-BA	1.00	1.00	1.00	0.28	0.16	0.20
AN-GD	1.00	1.00	1.00	0.51	0.35	0.39
MO-GU	0.71	0.56	0.50	0.50	0.34	0.73
MO-DO	0.62	0.45	0.31	0.56	0.39	0.84
MO-MA	0.42	0.27	0.08	0.93	0.87	0.98
MO-SL	0.26	0.15	0.04	0.52	0.36	0.81
MO-BA	0.33	0.20	0.11	0.88	0.79	0.96
MO-GD	1.00	1.00	1.00	0.89	0.80	0.94
GU-DO	0.26	0.15	0.09	0.18	0.10	0.06
GU-MA	0.69	0.53	0.49	0.45	0.29	0.26
GU-SL	0.28	0.16	0.40	0.38	0.24	0.13
GU-BA	0.67	0.50	0.52	0.65	0.48	0.54
GU-GD	1.00	1.00	1.00	0.77	0.62	0.73
DO-MA	0.58	0.41	0.30	0.36	0.22	0.16
DO-SL	0.25	0.14	0.22	0.36	0.22	0.12
DO-BA	0.53	0.36	0.33	0.73	0.57	0.56
DO-GD	1.00	1.00	1.00	0.76	0.61	0.74
MA-SL	0.35	0.21	0.03	0.33	0.19	0.18
MA-BA	0.19	0.10	0.03	0.85	0.74	0.86
MA-GD	1.00	1.00	1.00	0.59	0.42	0.74
SL-BA	0.24	0.14	0.05	0.77	0.62	0.71
SL-GD	1.00	1.00	1.00	0.54	0.37	0.75
BA-GD	1.00	1.00	1.00	0.39	0.24	0.67

T. bicolor

	Ensemble			Compound Community		
Pairwise comparisons	J_{ED}	S_{ED}	MH_{ED}	J_{CC}	S_{CC}	MH_{CC}
AN-MO	1.00	1.00	1.00	0.82	0.69	0.91
AN-GU	1.00	1.00	1.00	0.77	0.62	0.71
AN-DO	1.00	1.00	1.00	0.71	0.55	0.69
AN-MA	1.00	1.00	1.00	0.68	0.51	0.63
AN-SL	1.00	1.00	1.00	0.30	0.18	0.56
AN-BA	1.00	1.00	1.00	0.67	0.51	0.66
MO-GU	1.00	1.00	1.00	0.45	0.29	0.62
MO-DO	0.88	0.78	0.86	0.51	0.34	0.81
MO-MA	1.00	1.00	1.00	0.81	0.68	0.84
MO-SL	1.00	1.00	1.00	0.34	0.21	0.76
MO-BA	1.00	1.00	1.00	0.82	0.69	0.85
GU-DO	0.35	0.21	0.06	0.17	0.09	0.06
GU-MA	0.13	0.07	0.02	0.34	0.21	0.23
GU-SL	0.49	0.32	0.20	0.34	0.21	0.10
GU-BA	1.00	1.00	1.00	0.59	0.42	0.44
DO-MA	0.29	0.17	0.08	0.25	0.15	0.20
DO-SL	0.55	0.38	0.19	0.31	0.18	0.08
DO-BA	1.00	1.00	1.00	0.62	0.44	0.41
MA-SL	0.44	0.29	0.22	0.20	0.11	0.13
MA-BA	1.00	1.00	1.00	0.35	0.21	0.14
SL-BA	0.67	0.50	0.53	0.55	0.38	0.33

Appendix 2b. Chao-Jaccard (*J*), Chao-Sørensen (*S*), and Morisita-Horn (*MH*) dissimilarity matrices including data from the early 1990's only. "ED" subscripts denote ensemble dissimilarities, "CC" subscripts denote compound community dissimilarities minus data of the focal species.

C. flaveola

	Ensemble			Compound Community		
Pairwise comparisons	J_{ED}	S_{ED}	MH_{ED}	J_{CC}	S_{CC}	MH_{CC}
AN-MO	0.83	0.71	0.75	0.92	0.85	0.96
AN-GU	0.38	0.24	0.14	0.94	0.88	0.92
AN-DO	0.18	0.10	0.04	0.88	0.79	0.92
AN-MA	1.00	1.00	1.00	0.95	0.91	0.98
AN-SL	0.80	0.67	0.68	0.60	0.43	0.69
AN-BA	0.00	0.00	0.00	1.00	1.00	1.00
MO-GU	0.85	0.74	0.73	0.64	0.47	0.72
MO-DO	0.84	0.72	0.74	0.63	0.46	0.91
MO-MA	0.88	0.78	0.83	0.89	0.80	0.88
MO-SL	0.81	0.68	0.83	0.32	0.19	0.76
MO-BA	0.83	0.71	0.75	0.90	0.82	0.88
GU-DO	0.33	0.20	0.10	0.21	0.12	0.09
GU-MA	0.83	0.70	0.84	0.37	0.23	0.62
GU-SL	0.40	0.25	0.47	0.42	0.26	0.43
GU-BA	0.38	0.24	0.14	0.89	0.80	0.89
DO-MA	0.87	0.77	0.88	0.22	0.12	0.38
DO-SL	0.70	0.54	0.62	0.38	0.24	0.27
DO-BA	0.18	0.10	0.04	0.75	0.59	0.67
MA-SL	0.40	0.25	0.33	0.41	0.25	0.14
MA-BA	1.00	1.00	1.00	0.39	0.24	0.14
SL-BA	0.80	0.67	0.68	0.64	0.48	0.36

Loxigilla

	Ensemble			Compound Community		
Pairwise comparisons	J_{ED}	S_{ED}	MH_{ED}	J_{CC}	S_{CC}	MH_{CC}
AN-MO	0.84	0.72	0.77	0.88	0.79	0.93
AN-GU	0.92	0.85	0.93	0.79	0.66	0.54
AN-DO	0.92	0.85	0.94	0.76	0.61	0.54
AN-MA	1.00	1.00	1.00	0.85	0.75	0.80
AN-SL	0.70	0.53	0.69	0.72	0.57	0.61
AN-BA	1.00	1.00	1.00	0.56	0.39	0.22
MO-GU	0.71	0.56	0.50	0.50	0.34	0.73
MO-DO	0.62	0.45	0.31	0.56	0.39	0.84
MO-MA	0.42	0.27	0.08	0.93	0.87	0.98
MO-SL	0.26	0.15	0.04	0.50	0.33	0.72
MO-BA	0.33	0.20	0.11	0.92	0.85	0.95
GU-DO	0.26	0.15	0.09	0.18	0.10	0.06
GU-MA	0.69	0.53	0.49	0.45	0.29	0.26
GU-SL	0.36	0.22	0.40	0.35	0.21	0.12
GU-BA	0.67	0.50	0.52	0.64	0.47	0.49
DO-MA	0.58	0.41	0.30	0.36	0.22	0.16
DO-SL	0.34	0.20	0.23	0.35	0.21	0.12
DO-BA	0.53	0.36	0.33	0.72	0.56	0.54
MA-SL	0.43	0.28	0.09	0.41	0.26	0.20
MA-BA	0.19	0.10	0.03	0.93	0.86	0.86
SL-BA	0.35	0.21	0.12	0.82	0.69	0.70

T. bicolor

	Ensemble			Compound Community		
Pairwise comparisons	J_{ED}	S_{ED}	MH_{ED}	J_{CC}	S_{CC}	MH_{CC}
AN-MO	1.00	1.00	1.00	0.82	0.69	0.91
AN-GU	1.00	1.00	1.00	0.77	0.62	0.71
AN-DO	1.00	1.00	1.00	0.71	0.55	0.69
AN-MA	1.00	1.00	1.00	0.68	0.51	0.63
AN-SL	1.00	1.00	1.00	0.26	0.15	0.43
AN-BA	1.00	1.00	1.00	0.78	0.64	0.67
MO-GU	1.00	1.00	1.00	0.45	0.29	0.62
MO-DO	0.88	0.78	0.86	0.51	0.34	0.81
MO-MA	1.00	1.00	1.00	0.81	0.68	0.84
MO-SL	1.00	1.00	1.00	0.30	0.18	0.70
MO-BA	1.00	1.00	1.00	0.83	0.71	0.85
GU-DO	0.35	0.21	0.06	0.17	0.09	0.06
GU-MA	0.13	0.07	0.02	0.34	0.21	0.23
GU-SL	0.32	0.19	0.07	0.35	0.21	0.11
GU-BA	1.00	1.00	1.00	0.58	0.41	0.43
DO-MA	0.29	0.17	0.08	0.25	0.15	0.20
DO-SL	0.55	0.38	0.19	0.31	0.18	0.08
DO-BA	1.00	1.00	1.00	0.62	0.44	0.41
MA-SL	0.44	0.29	0.22	0.20	0.11	0.13
MA-BA	1.00	1.00	1.00	0.35	0.21	0.14
SL-BA	0.67	0.50	0.53	0.55	0.38	0.33