ORIGINAL ARTICLE

Haemosporidian parasites and avian host population abundance in the Lesser Antilles

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ABSTRACT

Aim To determine statistical relationships between the prevalence of haemosporidian (malaria) parasites and the abundance of avian host populations across islands in the Lesser Antilles.

Location Thirteen islands in the Lesser Antilles, from Grenada in the south to St Kitts in the north.

Methods Birds were captured by mist net and small blood samples were taken for polymerase chain reaction and DNA sequencing analysis of haemosporidian parasite infections. Similarities between islands with respect to geographical distance, avian host assemblages and haemosporidian parasite assemblages were compared by partial Mantel tests. Relative abundances of avian host populations across islands were compared to the relative abundances of common haemosporidian lineages by stepwise regression.

Results Populations of parasite lineages were more heterogeneously distributed than were host populations across the islands; parasite lineages frequently shift between hosts on different islands and exhibit range disjunctions. Relative population sizes of two widespread and common species of bird in the Lesser Antilles, the bananaquit *Coereba flaveola* and the black-faced grassquit *Tiaris bicolor*, were negatively related to the relative abundances of two parasite lineages, OZ01 (*Plasmodium* sp.) and OZ12 (*Haemoproteus* sp.), although the latter parasite was not recovered from either host. Positive associations between populations of *Elaenia martinica* and the relative abundance of three parasite lineages in populations of *Vireo altiloquus* additionally suggest the possibility of apparent competition between avian species mediated through haemosporidian parasites.

Main conclusions The results of these analyses are consistent with a strong influence of some avian haemosporidian parasites on populations of their hosts, including indirect interactions through apparent competition. In addition, the heterogeneity of host–parasite associations between islands suggests an evolutionarily dynamic system that is consistent with phases of host expansion and contraction, commonly known as the taxon cycle.

Keywords

apparent competition, *Haemoproteus*, host–parasite interaction, pathogen, *Plasmodium*, taxon cycle

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INTRODUCTION

The distribution and taxonomic differentiation of bird species in the Lesser Antilles are consistent with alternating phases of geographical expansion and contraction of the ranges of individual species over evolutionary time within the island archipelago (Ricklefs & Cox, 1972). This historical scenario has been confirmed by phylogeographical analyses

of individual species based on DNA sequences (e.g. Lovette *et al.*, 1998; Hunt *et al.*, 2001; Ricklefs & Bermingham, 2001; Joseph *et al.*, 2004; Miller *et al.*, 2007; Bellemain *et al.*, 2008). Because phases of expansion and contraction appear to be independent among similar species, Ricklefs & Cox (1972) doubted that they were caused by environmental drivers (cf. Smith *et al.*, 2012) and postulated that these 'taxon cycles' (Wilson, 1959, 1961) reflected the outcomes of coevolutionary interactions (actually, 'counter-evolutionary', because the interactions are antagonistic) of populations with their predators and pathogens.

The expression of these interactions at any particular time presumably is determined by mutations that arise infrequently and influence predator efficiency or pathogen virulence, on one hand, and the defenses of potential prey or resistance of affected hosts, on the other hand (Ricklefs, 2010a). When genetic factors favour a particular host, its population might increase locally and disperse more readily between islands. Alternatively, when this balance favours the pathogen (or predator), individual island populations of a host might decline and perhaps become extinct, and dispersal between islands might decrease, leading to differentiation of island populations. In this context, among species of West Indian birds, strong correlations are observed between geographical distribution, local abundance of island populations, and the breadth of habitats occupied, all of which decrease with progression through the taxon cycle after the initial expansion phase (Cox & Ricklefs, 1977; Ricklefs & Cox, 1978).

The ecological and evolutionary components of the taxon cycle model are understood in their general context. The depressing effects of disease organisms on host populations following introduction of pathogens to a new area are familiar (e.g. Van Riper *et al.*, 1986; Atkinson *et al.*, 1995; Hochachka & Dhondt, 2000; Hosseini *et al.*, 2006; Ricklefs, 2010b), as are rapid increases of host populations introduced to areas free of host-specific pathogens (Mitchell & Power, 2003; Reinhart *et al.*, 2003; Torchin *et al.*, 2003; Mitchell *et al.*, 2006; Inderjit & van der Putten, 2010; Hopper *et al.*, 2014). However, variations in local population sizes and geographical distributions of native host species rarely have been related to the impacts of native, endemic pathogens (cf. Gulland, 1995; Hudson *et al.*, 1998; Cavanaugh *et al.*, 2004), particularly in a community context, although such interactions likely are common (Ricklefs, 2011, 2013).

Here, we examine the relationship between the relative abundances of avian hosts and several lineages of avian haemosporidian parasites (Haemosporida: *Plasmodium* and *Haemoproteus*) on 13 islands in the Lesser Antilles, West Indies. These parasites have been shown in several studies to have pathogenic effects on their natural hosts (e.g. Ots & Hõrak, 1998; Merino *et al.*, 2000; Marzal *et al.*, 2005; Palinauskas *et al.*, 2008; Martínez-de la Puente *et al.*, 2010; Asghar *et al.*, 2011; Lachish *et al.*, 2011). We found that populations of two of the most common host species are inversely related across islands to the relative abundance of one or more haemosporidian parasite lineages, providing evidence consistent with negative effects of haemosporidian parasites on avian host populations. The statistical impact of parasites of one host species on populations of another host species also suggest that host species can interact through shared pathogens (apparent competition: Holt, 1977; Hudson & Greenman, 1998).

MATERIALS AND METHODS

Field methods

Our analysis is based on the relative abundances of eight common species of bird and 12 common lineages of haemosporidian ('malaria') parasite (Haemosporida: *Plasmodium* and *Haemoproteus*) on 13 islands in the Lesser Antilles, from St Kitts (SK) in the north, through Nevis (NE), Montserrat (MO), Barbuda (BU), Antigua (AN), Guadeloupe (GU), Dominica (DO), Martinique (MA), St Lucia (SL), Barbados (BA), St Vincent (SV), Carriacou (CA) and finally to Grenada (GR), in the south (Fig. 1). Details concerning the samples are provided in the Supporting Information. The Lesser Antilles are relatively isolated with respect to their avian populations. Few migrants from North America reach the islands; few non-native avian species have been introduced; recent passerine invasions of the island chain from South America involve only the shiny cowbird (*Molothrus bonairiensis*) and the bare-eyed thrush (*Turdus nudigenis*).
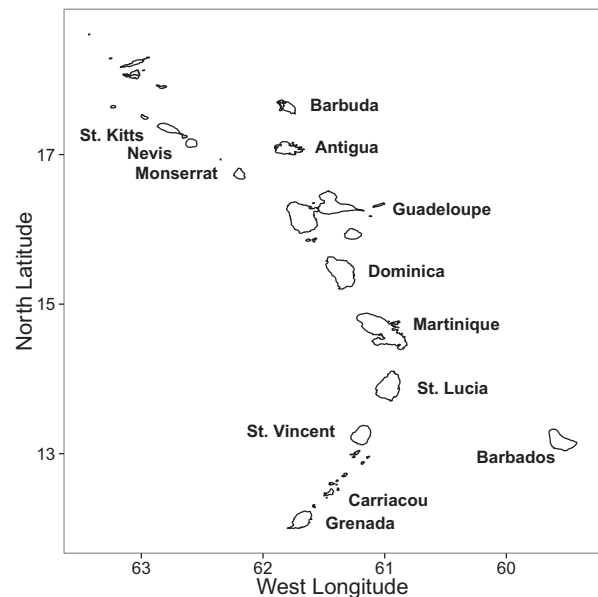

Figure 1 Map showing the sampled islands of the volcanic chain from St Kitts in the north to Grenada in the south. Barbuda, Antigua, the eastern half of Guadeloupe and Barbados are low-lying uplifted limestone platforms. Carriacou lies on a shallow bank between St Vincent and Grenada. Based on the Political Boundaries shapefile provided by the U.S. Geological Survey (http://pubs.usgs.gov/of/1997/ofr-97-470/OF97-470K/graphic/data.html).

On Trinidad, the cowbird is infected with lineages LA20 and LA24, and we recovered lineages OZ02 and OZ16 from three bare-eyed thrushes on GR; none of these lineages is common in the Lesser Antilles and they are not included in the present analysis.

We captured birds with mist nets in representative habitats on each island over periods of 2–8 days, generally during the late spring and summer months (see Appendix S1), which coincide with the early part of the rainy season in the Lesser Antilles. Although sampling occurred at different times of the year, over a span of more than 20 years across the islands (1991–2013), and during two periods separated by 9–15 years on BA, SL, SV, and GR, seasonal samples from a single location (Guanica Forest, south-west Puerto Rico) and samples obtained from the same island separated by periods of up to 15 years differed little compared to the differences between islands observed in this study (Fallon et al., 2004; Ricklefs et al., 2011). Thus, we believe that our samples represent, reasonably well for each island, the haemosporidian parasite communities as well as the host distribution and prevalence of individual parasite lineages.

Because netting effort varied across islands, we calculated the relative abundance of each species of avian host as the proportion of total mist net captures on a particular island. Thus, while capture effort varied between islands, netting techniques and habitats surveyed were similar, and the relative abundances of host species between islands are likely comparable by this sampling method (Blake & Loiselle, 2001). To validate the consistency among independent estimates of host population abundance, we compared the relative proportion of five of the more common species included in the present analyses with point-count estimates, based on identical protocols, of the abundances of the same species on SL and SK (Cox & Ricklefs, 1977), GR (Wunderle, 1985), and DO (I. J. Lovette, unpublished data). We used a general linear model (SAS GLM Procedure) to relate the logarithms of the relative proportions of each of five host species [bananaquit Coereba flaveola (CFA), Lesser Antillean bullfinch Loxigilla noctis (LNO), black-faced grassquit Tiaris bicolor (TBI), Lesser Antillean elaenia Elaenia martinica (EMA) (except on GR, where the species is rare), and black-whiskered vireo Vireo altiloquus (VAL)] to the logarithms of the point-count totals for each of the islands. Neither island nor species was a statistically significant effect ($P > 0.5$). With islands and species removed from the model, the common logarithm of the proportion of our net samples was positively correlated with the common logarithm of the point count for each species (Pearson correlation, $r_P = 0.75$, $P < 0.0001$; Spearman rank correlation, $r_S = 0.64$; $P = 0.002$). Thus, the proportional representation of a species in our mist net samples is generally related to the relative population size of the species on each island determined by point counts.

Blood samples (5–10 μL) were obtained from captured individuals by venipuncture from the brachial vein of one wing and stored in Puregene® (Germantown, MD, USA) or Longmire's (Longmire et al., 1997) lysis buffer. Captured individuals were generally held for < 15 min and all individuals were released unharmed immediately after processing (for further details of field methods, see Latta & Ricklefs, 2010). All samples were collected under IACUC protocols approved at the University of Pennsylvania (collections up to 1995) and the University of Missouri-St Louis (after 1995) and under appropriate permits from the governments of the individual islands, and were imported to the United States under permit from the Fish and Wildlife Service (USFWS) and the Animal and Plant Health Inspection Service (APHIS).

Laboratory methods

We extracted DNA from lysis buffer by alcohol precipitation following removal of proteins by ammonium acetate precipitation (Fallon et al., 2003a, 2005; Ricklefs et al., 2005). We screened DNA samples for the presence of haemosporidian parasites by polymerase chain reaction (PCR) amplification of a 154-bp segment of the mitochondrial SSU ribosomal DNA (Fallon et al., 2003b). We amplified and sequenced regions of the mitochondrial cytochrome b gene from positive samples using a variety of primer pairs and protocols (Bensch et al., 2000; Hellgren et al., 2004; Fallon et al., 2005; Ricklefs et al., 2005; Latta & Ricklefs, 2010; Outlaw & Ricklefs, 2010; Svensson-Coelho & Ricklefs, 2011). Our protocols sometimes fail to recognize mixed infections (often expressed as double peaks in sequence chromatograms), potentially leading to underestimation of parasite lineage prevalence in a host population. In addition, many infections detected by PCR and sequencing are missed on routine screening of blood smears, indicating very low parasitemia (Fallon & Ricklefs, 2008) and, possibly, infections that cannot be transmitted by dipteran vectors.

Lineage distinction

We distinguished lineages of haemosporidian parasites based on sequence differences and host and geographical distribution (Svensson-Coelho et al., 2013; Ricklefs et al., 2014). The parasites included in this analysis were two lineages of Plasmodium (represented in bold type, **OZ01** and **OZ04**), two lineages of Haemoproteus (subgenus Haemoproteus) (represented in italics, GA01 and GA02), recovered almost exclusively from the common ground dove Columbina passerina (CPA), and eight lineages of Haemoproteus (subgenus Parahaemoproteus): DR02, LA01, LA02, LA07, LA19, OZ12, OZ17 and OZ21 (see Appendix S2). The only named species of parasite that we know of among these are the widespread Plasmodium relictum (**OZ01**) and Haemoproteus coatneyi (OZ21). The minimum cytochrome b genetic distance between the lineages was 0.017 (GA01-GA02), and the mean and maximum genetic distances were 0.084 and 0.129. Of the lineages used in this analysis, DR02, LA02, GA01, GA02, LA07, LA19, **OZ04** and OZ21 are distributed primarily in

the West Indies; the other lineages are frequent in populations of migratory species in North America, as well.

Analysis

Pearson and Spearman correlations of relative abundances (proportions of all host captures and proportions of all parasite recoveries on individual islands) among the host and parasite populations across the islands were calculated by the SAS version 9.3 CORR Procedure (SAS Institute, Cary, NC, USA). The relationships among the island host and parasite faunas were determined by Bray–Curtis (BC) ordination and nonmetric multidimensional scaling (NMDS, using Sørensen distances) in PCOrd v.6 (MjM Software Design, Gleneden Beach, OR, USA), and the two ordinations were compared using canonical correlation (SAS CANCORR procedure). We tested the overall statistical significance of the canonical correlations between the host and parasite ordinations by comparing the observed Pillai's trace (the sum of the squared canonical correlations) to its distribution for the same analysis based on randomized matrices (9999 permutations), using the function *CCorA* in the 'vegan' package (Oksanen *et al.*, 2013) in R 3.1.2 (R Core Team, 2014).

We also created three between-island distance matrices comprising BC dissimilarities of host and parasite relative abundances across islands, using the *vegdist* function in the 'vegan' package, and geographical distances between islands, using the *rdist.earth* function in the 'fields' package (Nychka *et al.*, 2014) in R 3.1.2. We compared these distance matrices with Mantel tests and partial Mantel tests ('vegan' package, Oksanen *et al.*, 2013). Partial Mantel tests allow one to test the statistical significance of a correlation between two matrices while controlling for the effect of a third (Legendre & Legendre, 1998). Statistical significance of the Mantel test statistics was determined by permutation following Legendre & Legendre (1998; 9999 permutations per test).

The relationships between host and parasite relative abundances on each island were determined for each host species by stepwise regression with both forward and maxr variable selection (SAS STEPWISE procedure). Forward selection progressively adds variables to a model that results in the maximum increase in the explained variance (R^2); maxr selection finds the set of 1, 2, 3, ..., explanatory variables that maximize the explained variance. Arcsine-transformation of relative abundances had little effect on the results and these analyses are not reported.

RESULTS

Distributions of the 10 most common host species and the 12 most common parasite lineages across islands in our samples are presented in Tables S3a and S3b in Appendix S3. Only CFA and TBI were captured on all the islands included in the analysis. Among parasite lineages, only OZ21 was recovered from all the islands.

Most of the common parasites in our samples were recovered from more than one host species, although several lineages appear to be host specialists within the limits of our sample: LA07 on CFA; *GA01* and *GA02* on the common ground dove CPA; LA19 on the pearly-eyed thrasher *Margarops fuscatus* (MFT); and OZ12 on VAL (Table 1). Lineages LA01 and LA02 are specialized on birds of the thrasher genus *Margarops* in our sample. Lineages **OZ01**, **OZ04**, OZ17 and OZ21, which are widespread in North America and the Caribbean Basin, and DR02, which is widespread among Coerebinae (Burns *et al.*, 2014) in the West Indies, evidently are host generalists.

Correlations among host populations and among parasite populations

Simple Pearson correlations (r_P) among the relative abundances of the nine host populations across 13 islands revealed few strong relationships, only one of which was negative (values for Spearman correlation coefficients (r_S) were similar to the values of r_P; because 36 independent comparisons were calculated among the nine hosts, the appropriate

Table 1 The most abundant and widespread avian hosts in the Lesser Antilles (rows) along with the most abundant haemosporidian parasite lineages (columns) recovered from these hosts. Hosts and parasites are ordered by their numerical occurrence (relative abundance) in our samples.

	OZ21	**OZ04**	LA07	*GA01*	LA01	DR02	*GA02*	LA 02	**OZ01**	OZ17	OZ12	LA19
CFA	92	22	42	1					6			1
LNO	129	14	1			36		1	1	1		
TBI	45	22	1	1	1	8			3			
CPA	1			49	1		20		1		1	
VAL	5			1		1			1	12	10	
EMA	1	2								1		
DPE									1	2		
MFU	2	2			26	1		8	1			10
MFT		1			19	1		13				

Host species are: CFA, *Coereba flaveola*; LNO, *Loxigilla noctis*; TBI, *Tiaris bicolor*; CPA, *Columbina passerina*; VAL, *Vireo altiloquus*; EMA, *Elaenia martinica*; DPE, *Setophaga petechia*; MFU, *Margarops fuscus*; MFT, *Margarops fuscatus*.

P-value for significance at α = 0.05 should be 0.05/36, or *c.* 0.001):

1. *Coereba flaveola* and VAL, $r_P = -0.55$, $P = 0.043$ [VAL is particularly scarce or absent from GR and CA, where CFA represented more than 30% of the captures; note that VAL is the nearly exclusive carrier of lineage OZ12, to which CFA is negatively related (see below)], although this would not explain the absence of VAL from these southern islands; the species occurs further south on the South American mainland;

2. *Loxigilla noctis* and EMA, $r_P = +0.70$, $P = 0.005$ [both species are infrequent at the southern end of the Lesser Antilles (SV, CA, and GR)];

3. *Vireo altiloquus* and the yellow warbler *Setophaga petechia* (DPE), $r_P = +0.62$, $P = 0.019$ (both are infrequent at the southern end of the Lesser Antilles and on MO);

4. Scaly breasted thrasher *Margarops fuscus* (MFU) and MFT, $r_P = +0.87$, $P < 0.0001$ (MFU is absent on islands south of SV, although both species co-occur on most islands of the Lesser Antilles).

Statistically significant pairwise correlations among relative abundances of parasites across the 13 islands, among which only one of five was negative, were as follows (because 66 independent comparisons were calculated among the 12 lineages, the appropriate *P*-value for significance at α = 0.05 should be 0.05/66, or *c.* 0.001):

1. OZ21-LA07, $r_P = -0.52$, $P = 0.060$ (LA07 is absent from most of the islands, except GR, CA, and BU, where OZ21 is scarce);

2. **OZ01-OZ04**, $r_P = +0.75$, $P = 0.003$ [both are common on the four core islands of the Lesser Antilles (GU, DO, MA, SL)];

3. *GA01-GA02*, $r_P = +0.93$, $P = 0.0001$ (these two lineages are almost perfectly correlated, but geographically heterogeneous, and restricted to a single host CPA);

4. LA01-LA02, $r_P = +0.83$, $P = 0.0003$ (both are absent from the southern islands);

5. DR02-OZ17, $r_P = +0.94$, $P = 0.0001$ (both are absent from the southern islands and are most frequent on BU, AN and NE).

Of the 108 simple pairwise correlations between the relative abundances of nine host species and 12 parasite lineages, seven combinations had both r_P and r_S statistically significant individually at $P < 0.01$, among which only one was negative (TBI and **OZ01**; $r_P = -0.66$, $P = 0.01$). The six positive correlations associated CPA with GA01 ($P < 0.0001$), both MFU and MFT with LA01 ($P < 0.0001$) and LA02 ($P < 0.001$), and EMA with OZ17 ($P < 0.009$).

Relationships of host and parasite distributions across islands

Host and parasite distributions across islands were independently analysed with BC and NMDS ordinations based on Sørenson dissimilarities calculated from the relative abundances of host species and the relative abundances of parasite lineages on each island. Each ordination produces axis scores for each of the islands, as well as for each of the host species or parasite lineages. Both the BC and NMDS ordinations were constrained to three axes. Canonical correlations between the two sets of scores for both the hosts and the parasites were nearly perfect: hosts, $P < 0.0001$, < 0.0001, 0.0005 for the three canonical axes; parasites, $P < 0.0001$, < 0.0001, 0.002. Subsequent analyses are based solely on the NMDS axis scores.

Distributions of islands and either hosts or parasites in ordination space were compared using their positions on the three NMDS ordination axes. The standard deviations of the positions of the islands on the three axes were similar whether ordinated by host species [standard deviation (SD) = 0.81, 0.51, 0.41] or by parasite lineages (SD = 0.78, 0.51, 0.47). However, positions of host species in the ordination space were generally more similar across islands (SD = 0.30, 0.19, 0.15) than were the positions of parasites (SD = 0.43, 0.34, 0.31), as is evident in the raw data from the more homogeneous distribution of hosts (see Table S2) compared to that of parasites (see Table S3).

The NMDS ordination axis scores for each of the islands based on hosts versus parasites were compared using canonical correlation to determine the extent to which the geographical structure of parasite assemblages paralleled that of their hosts. None of the three canonical correlations were statistically significant individually: $R^2 = 0.68$, 0.39, and 0.21 ($P = 0.077$, 0.18, and 0.15). However, Pillai's trace (the sum of the squared canonical correlations, 1.27) differed significantly from random ($P = 0.027$) indicating some degree of concordance between the differentiation of host and parasite assemblages across islands.

A Mantel test revealed a weak, but statistically significant correlation between host and parasite dissimilarities between islands ($r = 0.37$, $P = 0.007$), accounting for about $r^2 = 0.14$ proportion of the variance. These dissimilarities were also correlated with geographical distance between islands for both parasites ($r = 0.38$, $P = 0.004$) and hosts ($r = 0.27$, $P = 0.025$). A partial Mantel test controlling for geographical distances between islands still showed a statistically significant relationship between host and parasite dissimilarities ($r = 0.30$, $P = 0.02$). Interestingly, while a partial Mantel test controlling for host dissimilarities revealed an association between parasite dissimilarities and geographical distances between islands ($r = 0.32$, $P = 0.01$), host dissimilarities were not found to be related to geographical distances when controlling for parasite dissimilarities ($r = 0.14$, $P = 0.10$). These results reflect the stronger geographical structure in the parasite populations compared to their hosts (see Tables S2 and S3). The generally weak correlations between the host and parasite assemblages across islands suggest that assemblages on each of the islands can be treated, for the purposes of this analysis, as statistically independent samples.

Statistical associations between host populations and individual parasite lineages

We conducted stepwise regressions relating the relative abundance of each host population to the relative abundances of all the parasite lineages on the same islands using the forward selection criterion in SAS Proc Stepwise (results based on maxr selection were similar, as were results based on arcsine square-root transformation of the relative abundances). For CFA, the final regression ($F = 15.2$, d.f. = 3.9, $P = 0.0007$, $R^2 = 0.84$) identified the following statistically significant associations with three parasite lineages: LA07, $+0.35 \pm 0.06$ ($P = 0.0004$); **OZ01**, -0.95 ± 0.39 ($P = 0.037$) (see Fig. 2); OZ12, -0.85 ± 0.24 ($P = 0.007$). Lineage LA07 (positive relationship) is common and is recovered almost exclusively from CFA; **OZ01** (negative relationship) is not a common parasite, but CFA is the most frequent host in the Lesser Antilles; OZ12 (negative relationship) is recovered almost exclusively from VAL, to which CFA is negatively related (see above); however, no OZ12 infections were identified from CFA in the Lesser Antilles.

For LNO, the final regression ($F = 10.3$, d.f. = 2.10, $P = 0.004$, $R^2 = 0.67$) identified the following positive associations with two parasite lineages: OZ21, $+0.19 \pm 0.05$ ($P = 0.002$); DR02, $+0.24 \pm 0.08$ ($P = 0.013$). Note that OZ21 is common in LNO, and LNO is the most frequent host of DR02 in the Lesser Antilles. *Loxigilla noctis* additionally exhibits a simple negative correlation with LA07, due largely to the absence of this host from CA, on which LA07 is the most prevalent parasite. For TBI, the final stepwise regression ($F = 8.7$, d.f. = 1,11, $P = 0.013$, $R^2 = 0.44$) included only **OZ01**: -1.27 ± 0.43, $P = 0.013$ (see Fig. 2). **OZ01** is infre-

quent in the Lesser Antilles: only three infections were identified in TBI and six in CFA out of a total of 18.

The common ground dove CPA was associated only with lineage *GA02* ($+1.23 \pm 0.17$, $P < 0.0001$, $R^2 = 0.83$), for which CPA is the sole host, as it is for *GA01*, whose distribution parallels that of *GA02* almost perfectly. VAL and DPE populations were not related to the relative abundances of any of the parasite lineages. Populations of MFU were positively related to the relative abundance of lineage LA02 ($+0.88 \pm 0.01$, $P < 0.0001$, $R^2 = 0.85$, $F = 63$, d.f. = 1.11), which is practically restricted to MFU, but not common overall. Populations of MFT were positively related to the relative abundance of lineage LA01, which was commonly recovered from this host species. Finally, populations of EMA were positively associated with the relative abundances of OZ21 (0.10 ± 0.03, $P = 0.008$), OZ17 ($+1.16 \pm 0.24$, $P = 0.001$) and OZ12 (0.56 ± 0.16, $P = 0.007$) ($F = 15.4$, d.f. = 3.9, $P = 0.0007$, $R^2 = 0.84$), although few parasites were recovered from this host species.

Table 2 presents two striking patterns. First, the three negative associations between hosts and parasites (CFA with **OZ01** and OZ12, and TBI with **OZ01**) involve lineages that are infrequent (**OZ01**) or absent (OZ12) from the affected hosts in our samples. Although **OZ01** was recovered primarily from CFA and TBI, it represents fewer than 5% of the infections of these host species; OZ12 is one of the two lineages frequently recovered from VAL. Second, the three parasite lineages positively associated with populations of EMA (all *Haemoproteus*) are infrequent in that species, but comprise 90% of the lineages recovered from VAL. Finally, both lineages LA01 and LA02 are commonly recovered from MFU and MFT, but each is associated with the abundance of only one of the two hosts.
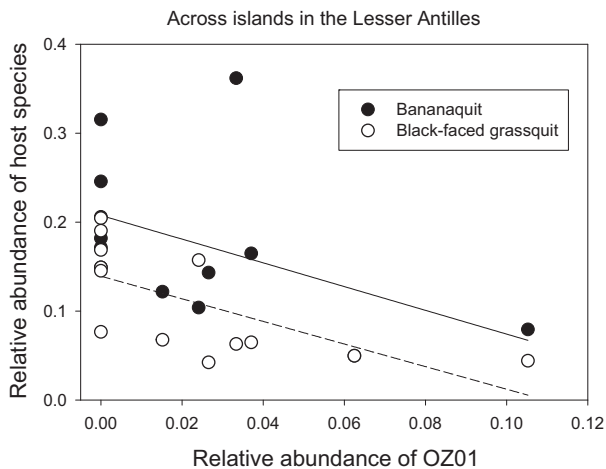

Figure 2 Negative relationship between the relative abundances of the bananaquit (*Coereba flaveola*) (CFA) and black-faced grassquit (*Tiaris bicolor*) (TBI), and the relative abundance of haemosporidian parasite lineage **OZ01**, on islands in the Lesser Antilles. The linear regression equations were CFA = 0.21 (± 0.02 SE)-1.34 (± 0.75) **OZ01** and TBI = 0.14 (± 0.02)-1.27 (± 0.43) **OZ01**.

DISCUSSION

We analysed the distributions and abundances of avian host species and their haemosporidian (malaria) parasites across islands in the Lesser Antilles to assess support for the hypothesis that pathogens can depress their avian host populations under natural conditions. The distributions of host and parasite populations across the island chain were related by comparing ordination scores for the islands with respect to both hosts and parasites, and by Mantel tests of the between-island host and parasite dissimilarity matrices. Neither analysis revealed strong relationships between host and parasite assemblages across islands, reflecting considerable independence of parasite distributions against a mostly similar set of host species. This difference in community structuring of hosts and parasites was reinforced by partial Mantel tests, which revealed no distance decay (Nekola & White, 1999) in the host fauna when parasite distributions were accounted for, but statistically significant distance decay in the parasite fauna when hosts were accounted for. This suggests that parasite distributions might be more dynamic locally than host distributions, potentially owing to more

Table 2 Distribution of the effects of haemosporidian parasite lineage relative abundance (rows) on avian host relative abundance (columns) across islands (regression coefficients). The most common hosts of each lineage in this table are indicated by grey shading. (+) and (−) indicate statistically significant positive and negative simple correlations between the relative abundances of hosts (Table S2) and parasites (Table S3) across islands.

	CFA	LNO	TBI	EMA	VAL	MFU	MFT
DR02		+0.24		(+)			
GA01							
GA02							
LA01						(+)	+0.34 (+)
LA02						+0.88 (+)	(+)
LA07		(−)					
LA19	+0.35						
OZ01	−0.95		−1.27 (−)				
OZ04							
OZ12	−0.95			+0.56			
OZ17				+1.16 (+)			
OZ21		+0.19 (+)		+0.10			

CFA, *Coereba flaveola*; LNO, *Loxigilla noctis*; TBI, *Tiaris bicolor*; VAL, *Vireo altiloquus*; EMA, *Elaenia martinica*; MFU, *Margarops fuscus*; MFT, *Margarops fuscatus*.

frequent population extinction and limited island recolonization compared to their avian hosts (Fallon *et al.*, 2004).

The heterogeneous distribution of parasite lineages among host species across islands and the shifting of some parasite lineages between hosts across islands (e.g. Ricklefs *et al.*, 2014) also suggest that parasite populations might interact through the immune systems of their hosts (Fig. 3). In a similar analysis of haemosporidian distributions in the Vanuatu Islands of the southwest Pacific Ocean, assemblages of avian *Plasmodium* lineages exhibited statistically significant distance effects but *Haemoproteus* lineages did not (Olsson-Pons *et al.*, 2015), suggesting that the geographical structuring of haemosporidian assemblages on islands might differ between archipelagos.

With respect to statistical associations between host and parasite populations in this analysis, we found that the relative abundances of two of the commonest host species in the Lesser Antilles, the bananaquit CFA and the black-faced grassquit TBI, which are closely related phylogenetically in the subfamily Coerebinae (Burns *et al.*, 2014), are negatively related to the frequency of one or two lineages of haemosporidian parasite [**OZ01** (Fig. 2) and OZ12] that are rarely or never recovered from these hosts. This suggests that these parasite lineages might be virulent and depress host populations through apparent competition between hosts mediated by shared pathogens. Lineage **OZ01**, which is a common parasite of a variety of emberizid species in North America (Ricklefs *et al.*, 2005) was recovered sporadically from a number of other host species in the Lesser Antilles (Table 1), but never in large numbers. At most **OZ01** comprised 11% of the parasites sampled on an island, but generally < 5%, and the lineage was absent from our samples on six of 13 islands, where TBI and CFA were particularly abundant. Although this parasite might exist in unsampled host reservoirs, we did capture most of the resident species of small land birds on each of the islands. Lineage OZ12 was recovered only from VAL in the Lesser Antilles, but it is also common in red-eyed vireos (*Vireo olivaceus*) in the
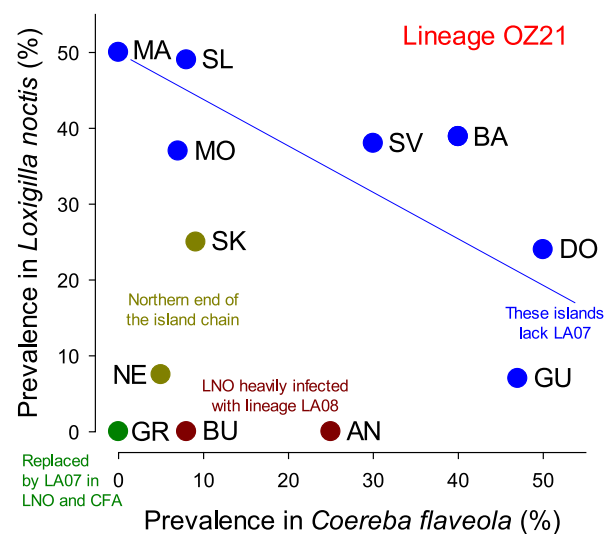

Figure 3 Inverse relationship of the prevalence of *Haemoproteus coatneyi* (OZ21) in the Lesser Antillean bullfinch (*Loxigilla noctis*) (LNO) and bananaquit (*Coereba flaveola*) (CFA) across islands in the Lesser Antilles. Note that Dominica (DO) and Guadeloupe (GU) apparently lack LA07, which is a common lineage in the bananaquit elsewhere but apparently replaced by OZ21 on these islands; bullfinch populations on Barbuda (BU) and Antigua (AN) are heavily infected with lineage DR02 but lack OZ21; on Grenada (GR), OZ21 is replaced in both host species by lineage LA07; OZ21 is also replaced by LA07 on Carriacou, but the bullfinch is missing from this island.

Midwestern United States (Ricklefs *et al.*, 2005), and was obtained from wintering red-eyed vireos in Trinidad (R. E. Ricklefs, unpublished data).

Positive associations between host abundance and parasite abundance (LNO with DR02 and OZ21, and MFU with LA22) might simply reflect the fact that these species are common hosts for these parasite lineages, and so the two vary in parallel, even if the host population is controlled by other factors. In contrast, the positive association of EMA with three

parasite lineages commonly recovered from VAL (OZ12, OZ17, and OZ21) is more difficult to explain. OZ12 is a common parasite of *V. olivaceus* in southern Missouri; OZ17 is also common in *V. olivaceus* in Missouri and has been recovered on the wintering grounds of this species in northern South America (R. E. Ricklefs, unpublished data); OZ21 is a parasite with a broad host distribution, but primarily limited to the Lesser Antilles and to Puerto Rico, just to the north; we have recorded only four instances from North America. *Elaenia martinica*, which is a small flycatcher (Tyrannidae), might benefit from these parasites if they reduced populations of one or more common competitors, but no negative correlations were detected between populations of EMA and any other host species, including VAL. Possibly, exposure and immune response to these parasite lineages might afford EMA protection against other pathogens, but we have no information bearing on such a mechanism.

Considering the large number of parasites and pathogens that potentially could influence populations of their hosts, the negative relationships observed in this analysis between the populations of several species of Lesser Antillean birds and their haemosporidian (malaria) parasites suggest that pathogen suppression of host populations might be a general mechanism accounting for variation in the relative abundance of populations in the absence of other obvious environmental influences. The sizes of some avian populations vary dramatically between islands without clear ecological explanations. For example, the absence of LNO from suitable habitats on CA Island in the Grenadines, where both CFA and TBI are common, begs explanation by a specialized cause that affects only that species. The absence of CPA, one of the most abundant birds in the West Indies, from our samples on BU and St Lucia would also be difficult to explain on the basis of variation in general ecological conditions. Regardless of these 'anomalies' in avian populations, haemosporidian parasite lineages are even more heterogeneously distributed across islands, independently of the presence of suitable host populations. Other, related lineages of *Plasmodium* and *Haemoproteus* are being transmitted on all of the islands, suggesting that vector limitation is not likely responsible for these patterns (but see Ricklefs *et al.*, 2011, for a possible case of vector limitation on haemosporidian parasites).

The possibility of pathogen influence on avian populations, indicated by statistical analyses of host abundance and parasite abundance, suggests that focused studies involving experimental infection of captive host individuals would be worthwhile. Because of logistical and ethical considerations with respect to Lesser Antillean birds, such studies are unlikely to be undertaken on a large scale in this region. However, additional surveys of pathogens with potential host population impacts are warranted and might contribute to our understanding of geographical variation in host population density and of factors responsible for phases of population expansion and contraction through the Lesser Antillean island chain.

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SUPPORTING INFORMATION

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

Appendix S1 Samples used in the analyses.
Appendix S2 Lineages of haemosporidian parasites included in this analysis.
Appendix S3 Samples of host species and parasite lineages across the archipelago.

BIOSKETCHES

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