Dynamics of avian haemosporidian assemblages through millennial time scales inferred from insular biotas of the West Indies

Leticia Soares^{a,1,2}, Steven C. Latta^b, and Robert E. Ricklefs^{a,1}

^aDepartment of Biology, University of Missouri–St. Louis, St. Louis, MO 63121-4499; and ^bNational Aviary, Pittsburgh, PA 15212

Contributed by Robert E. Ricklefs, May 4, 2017 (sent for review February 14, 2017; reviewed by Andrés Baselga and Fabien Leprieux)

Although introduced hemosporean (malaria) parasites (Apicomplexa: Haemosporida) have hastened the extinction of endemic bird species in the Hawaiian Islands and perhaps elsewhere, little is known about the temporal dynamics of endemic malaria parasite populations. Haemosporidian parasites do not leave informative fossils, and records of population change are lacking beyond a few decades. Here, we take advantage of the isolation of West Indian land-bridge islands by rising postglacial sea levels to estimate rates of change in hemosporean parasite assemblages over a millennial time frame. Several pairs of West Indian islands have been connected and separated by falling and rising sea levels associated with the advance and retreat of Pleistocene continental glaciers. We use island isolation following postglacial sea-level rise, ca. 2.5 ka, to characterize long-term change in insular assemblages of hemosporean parasites. We find that assemblages on formerly connected islands are as differentiated as assemblages on islands that have never been connected, and both are more differentiated than local assemblages sampled up to two decades apart. Differentiation of parasite assemblages between formerly connected islands reflects variation in the prevalence of shared hemosporean lineages, whereas differentiation between islands isolated by millions of years reflects replacement of hemosporean lineages infecting similar assemblages of avian host species.

avian malaria | bananaquit | beta diversity | *Plasmodium* | *Haemoproteus*

Insular biotas provide natural laboratories for characterizing change in host–parasite relationships over time (1). Haemosporidian parasites (genera *Plasmodium* and *Haemoproteus*, among others) are dipteran vector-transmitted protozoans that reproduce clonally in cells of vertebrates and cause symptoms referred to as malaria (2). The few fossils of ancient hemosporeans (3) are insufficient to analyze retrospectively the dynamics of these host–parasite interactions. We have found that hemosporean parasite assemblages of birds present remarkable geographic heterogeneity in the West Indies, even though common avian host species are widely distributed throughout the archipelago (4). Additionally, the frequencies of individual lineages in assemblages of avian hemosporean parasites have been observed to vary over periods as short as one decade (5–7). Here, we take advantage of the geographic history of islands in the West Indies to characterize changes in avian hemosporean parasite assemblages over millennial time scales.

During the late Quaternary glaciations, which reached their maximum extent ca. 26 ka, an increase in continental ice volume caused the global sea level to drop by as much as 120 m, exposing land connections between islands lying on shallow banks (8). During periods of sea level lows, organisms could freely disperse between some pairs of present-day islands, tending to homogenize assemblages of birds and, presumably, their pathogens (8). Deglaciation started 7.5–10 ka, and ended ca. 2.5 ka when seas returned to currently observed levels and many pairs of islands were reisolated (9) (Fig. 1). We use island coalescence during glacial sea-level lows to define natural experimental groups, in

each of which host and parasite assemblages presumably were homogenized and then subsequently isolated, providing ca. 2.5 ka during which insular host–parasite assemblages could change independently and diverge.

We ask whether the period from the reisolation of insular biotas by rising sea levels to the present has been sufficient for the homogenized parasite assemblages on the previously connected island pairs to become differentiated. We quantify differences in host and parasite assemblages by indices based on differences in parasite prevalence, as well as by gains and losses of hemosporean lineages on one island of a pair relative to the other. Finally, we ask whether differences in assemblage composition are due to replacement of lineages that are more or less closely related through recent common ancestry than one would expect of random replacement.

To establish relative points of comparison with the millennial time frame of hemosporean assemblage isolation, we compare parasite assemblages from islands that were formerly connected and assemblages from islands separated by deep-water channels for millions of years. These island pairs are relatively well matched in terms of size and ecology. We account for short-term variation by comparing parasite assemblages from the same island sampled between time intervals as short as 2 y and as long as two decades. Finally, we use pairwise comparisons between assemblages sampled at the same time, but at different locations within the same island, to assess parasite assemblage homogenization over distance and the assumption, implicit in our analysis, that assemblages were initially homogeneous across formerly connected islands. Assemblage comparisons, sample size, and year of collection are listed in

Significance

To study long-term changes in ecological assemblages in the absence of a fossil record, we take advantage of the joining and separation of islands on shallow banks in the West Indies archipelago caused by glacial-cycle sea-level changes. We compare pairs of assemblages of avian malaria parasites on islands that were connected during periods of low sea levels and subsequently isolated about 2.5 ka with assemblages on islands that were never connected, as well as assemblages sampled over local scales of time and space on a single island. This approach reveals millennial-scale change in the prevalence of parasite lineages followed by lineage replacement over longer periods, providing unique access to long-term change.

Author contributions: L.S., S.C.L., and R.E.R. designed research; L.S., S.C.L., and R.E.R. performed research; L.S. analyzed data; and L.S., S.C.L., and R.E.R. wrote the paper.

Reviewers: A.B., University of Santiago de Compostela; and F.L., Université de Montpellier.

The authors declare no conflict of interest.

¹Present address: Department of Biology, Saint Louis University, St. Louis, MO 63103.

²To whom correspondence may be addressed. Email: ricklefs@umsl.edu or leticiassoares@gmail.com.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1702512114/-DCSupplemental.

Fig. 1. Bathymetric map depicting Puerto Rico and islands of the Lesser Antilles in the West Indies. Black contours delineate the 100-m isobath (depth contour). This isobath approximates shorelines at the most recent sea level minimum, ca. 26 ka. Islands that were formerly connected, as well as their isolated counterparts, are labeled; the Cayman Islands are located to the west in the Greater Antilles, outside the map area. Bathymetry data were obtained from the National Oceanic and Atmospheric Administration.

SI Appendix, Tables S1 and S2. We analyze two types of parasite assemblages. Island assemblages refer to all hemosporidian lineages sampled on an island. Host focal assemblages refer to hemosporidian lineages recovered from a suite of six common bird species across individual islands. We compare bird assemblages themselves to quantify the influence of variation in host composition on changes in hemosporidian assemblages over time.

Our analyses are based on blood samples obtained from 6,270 individuals of 143 avian host species across 17 islands of the West Indies archipelago, five pairs of which were connected until ca. 2.5 ka (Fig. 1 and *SI Appendix, Tables S1 and S2*). Parasite assemblage data come from 1,245 sequenced hemosporidian infections assigned to 71 phylogenetically distinct lineages, which are defined by nucleotide differences in a region of the parasites' mitochondrial cytochrome *b* gene (10). Beta (β) diversity is a between-sample component of variance used by community ecologists to quantify differences between two or more assemblages of organisms. Here, β diversity metrics are used to describe differences in the composition of hemosporidian parasite assemblages between pairs of islands, and over space and time within islands. We use these metrics to compare four groups of assemblage pairs associated with distinct points on a time scale of island isolation: (i) islands never connected, and separated by millions of years; (ii) islands connected until ca. 2.5 ka; (iii) assemblages from the same island, but sampled over intervals of 2 y to two decades; and (iv) contemporary assemblages from the same island, isolated by distances comparable to the distances between islands, which tests the assumption of homogeneity between pairs of assemblages connected by land.

Results

We found that parasite assemblages on islands connected by land bridges until ca. 2.5 ka were as differentiated as pairs of assemblages on islands that have never been connected, and assemblages in both groups of comparisons were considerably more differentiated than assemblages from the same island

sampled at intervals of up to two decades, or between contemporaneous samples from the same island ($\beta_{\text{Chao-Sørensen}}$ island assemblages: $\chi^2 = 31.8$, $P < 0.01$; host focal assemblages: $\chi^2 = 6.0$, $P = 0.04$; Fig. 2 and *SI Appendix, Tables S3A and S4*). Avian host assemblages are not differentiated within these comparisons ($\beta_{\text{Chao-Sørensen}}$ avian assemblages: $\chi^2 = 3.9$, $P = 0.2$; *SI Appendix, Table S4*); most of the common host species included in our analyses are widely distributed within the West Indies (11). Thus, the increasing dissimilarity between hemosporidian assemblages over time is not caused by changes in host assemblages.

β diversity quantifies the difference between two assemblages, and it can be partitioned into components that represent replacement of parasite lineages (i.e., coupled gains and losses), differential gains or losses of lineages, and variation in lineage prevalence (11). Each partition might represent assembly processes that occur on distinct time scales. Hemosporidian lineages often present short-term variation in prevalence (5–7), including cycles in the proportion of infected host individuals (5). Replacement of lineages might reflect sorting processes that constrain assemblage composition, including competition among parasite lineages, involving interactions through the host immune system and the evolution of host resistance.

Most parasite lineages on individual islands were replaced or lost over periods of 2.5 ka or more (host focal assemblages, $\beta_{\text{Replacement}}$: $\chi^2 = 9.3$, $P < 0.01$; $\beta_{\text{Nestedness}}$: $\chi^2 = 8.2$, $P < 0.01$; Fig. 2 and *SI Appendix, Table S4*). Lineages within host focal assemblages were replaced almost twice as often between islands isolated for millions of years than between islands separated by ca. 2.5 ka. However, lineages were replaced between formerly connected assemblages more frequently than between local assemblages sampled over time intervals of up to two decades. Parasite lineage replacement in host focal assemblages was not related to replacement of host species, which do not vary significantly across comparison groups ($\beta_{\text{Replacement}}$ avian assemblages: $\chi^2 = 5.4$, $P = 0.07$; *SI Appendix, Table S4*). The overall lineage composition within an island appeared to be stable, because we observed no differences in the frequencies with which lineages were replaced between island assemblages across all comparison groups ($\beta_{\text{Replacement}}$: $\chi^2 = 2.9$, $P = 0.4$; $\beta_{\text{Nestedness}}$: $\chi^2 = 2.3$, $P = 0.5$; *SI Appendix, Table S4*), even though replacement in host focal assemblages was more frequent at longer time intervals. Hence, changes in lineage prevalence, not replacement or losses of lineages, are primarily responsible for the decrease in similarity with increasing isolation time in island assemblages. Thus, although the composition of the hemosporidian assemblage of an island remains stable through time, parasite lineages appear to switch among different host species within an island. The observed temporal stability of insular hemosporidian assemblage composition is likely a result of limited parasite dispersal among islands within the archipelago, because differential gains and losses of lineages ($\beta_{\text{Nestedness}}$) did not explain differentiation of assemblages with increased isolation time (12) (*SI Appendix, Table S4*).

Finally, we ask whether replacement of hemosporidian lineages involves closely or distantly related parasites. If closely related lineages replaced one another more often than expected by chance, parasite assemblages might be constrained by one or more traits that are shared by common descent through parasite evolutionary history. Alternatively, if substituted lineages are distant relatives, competitive exclusion might constrain the coexistence of closely related lineages within an island host fauna. We found that gain and loss of lineages over evolutionary time causes increasing phylogenetic divergence of hemosporidian assemblages between islands isolated by progressively greater time intervals ($\beta_{\text{Phylo-Sor PD}}$: $\chi^2 = 7.7$, $P = 0.02$; *SI Appendix, Table S4*), where *PD* specifies phylogenetic diversity of an assemblage. However, replacement of hemosporidian lineages apparently is unrelated to phylogenetic relationships among parasites

Fig. 2. Haemosporidian assemblage dissimilarity across groups of island pairwise comparisons. Dissimilarity is represented by two metrics: Chao-Sørensen (i.e., overall dissimilarity describing differences in lineage composition and prevalence) and Sørensen-Replacement (i.e., dissimilarity describing differences in lineage composition only), which are summarized for each group of pairwise assemblage comparisons. The Chao-Sørensen metric estimates dissimilarity between two assemblages based on (i) the prevalence of parasite lineages, (ii) the number of lineages shared between two assemblages, and (iii) the number of unique lineages in each assemblage. Chao-Sørensen values vary from 0 for identical assemblages to 1 for assemblages that differ completely with respect to one metric component or a combination of the metric components. Sørensen-Replacement is a compositional dissimilarity metric that measures only the sharing, or not, of lineages and attributes zero weight to differences in prevalence. When two assemblages of hosts support the same lineages of parasites, Sørensen-Replacement = 0; when no lineages are shared, Sørensen-Replacement = 1. (Left) Box plots are used to summarize dissimilarity metrics for pairwise comparisons based on the overall haemosporidian assemblage within each sample. Between island haemosporidian assemblages, dissimilarity measured by Chao-Sørensen (Top) increases with isolation time ($\chi^2 = 31.8$, $P_{\chi^2} < 0.01$), but replacement of lineages (Bottom) is unrelated to assemblage isolation ($\chi^2 = 2.9$, $P_{\chi^2} = 0.4$). (Right) Summary of dissimilarity metrics for pairwise comparisons based on haemosporidian assemblages of bananaquits (*C. flaveola*). (Top) Similar to island assemblages, these metrics show a pattern of decay in assemblage similarity with isolation time based on the Chao-Sørensen dissimilarity metric ($\chi^2 = 6.0$, $P_{\chi^2} = 0.04$). (Bottom) Replacement of haemosporidian lineages in bananaquit assemblages increases with isolation time ($\chi^2 = 9.3$, $P_{\chi^2} < 0.01$), however. Contemporary comparisons of haemosporidian assemblages from bananaquits were not included in the analyses due to sampling limitation.

($\beta_{\text{Phylo-Sor Replacement}}$, island assemblages: $\chi^2 = 4.8$, $P = 0.2$; host focal assemblages: $\chi^2 = 3.2$, $P = 0.2$; *SI Appendix, Table S4*). This result mirrors the observation that the hosts of related parasites (i.e., sister lineages) are not more closely related than pairs of species drawn at random from the host taxonomic hierarchy (13). In the West Indies, we frequently observe locally prevalent and geographically widespread lineages replacing one another across islands of the archipelago (12, 14) (Fig. 3).

Discussion

We found that pairs of insular haemosporidian assemblages that were formerly homogenized, but isolated for the past *ca.* 2.5 ka, differ to an extent comparable to pairs of assemblages that have never been connected by land (Fig. 2). Such differentiation of parasite assemblages takes place independently of possible contemporary overwater dispersal of avian hosts across islands of the archipelago. The most detailed retrospective record of endemic haemosporidian assemblages is from a breeding population of great reed warbler (*Acrocephalus arundinaceus*) from Scandinavia (5). Over 17 y, *A. arundinaceus* hosts were mainly infected by three haemosporidian lineages that presented coupled periodicity in prevalence, with cycles lasting 3–4 y (5). On Puerto Rico and Saint Lucia, in the West Indies, changes in haemosporidian assemblages over intervals of approximately 10 y were mostly due to variation in the prevalence of parasite lineages, but gains and losses of lineages were also observed (7). Similar patterns are observed for human malaria parasites. A 14-y cohort study of *Plasmodium falciparum* infections of humans in Brazil revealed temporal fluctuations in the frequency of allelic variants of the polymorphic merozoite surface protein gene (MSP-1) (15). Mathematical models have suggested that parasite prevalence can present periodic and even chaotic behavior when host populations are exposed to pathogens with polymorphic antigens

(16). Although we present strong evidence of temporal variation over evolutionary time scales (Fig. 2 and *SI Appendix, Table S4*), the mechanisms producing change in haemosporidian assemblages remain unknown.

Our results suggest long-term temporal variation in the regulatory effects of haemosporidian parasites on their host populations, and vice versa. In the initial stages of a host–parasite interaction, parasites exert a strong selective pressure, and therefore a regulatory effect, over their host populations (i.e., periods of high parasite prevalence). Through time, host susceptibility decreases as hosts evolve immune resistance, switching the direction of selective pressure toward parasite populations and reducing parasite prevalence (17). Haemosporidian parasites can depress avian populations on islands of the Lesser Antilles, where relative population sizes of two widespread and common species of bird were negatively related to the relative abundances of two common parasite lineages (14). Hence, temporal change in the prevalence of endemic parasite lineages in the West Indies and elsewhere could reflect host–parasite evolution and coevolution.

Islands in the West Indies exhibit stability in the composition of their parasite assemblages, as shown by the low frequency of lineage replacement through time. However, haemosporidian assemblages of individual host species within an island are more dynamic, resulting from switching of parasite lineages between host species, which is apparent over time scales of several thousands of years. This result suggests that differences in haemosporidian parasite–avian host assemblages between islands are due to host switching of parasite lineages between host populations within an island, rather than replacement of lineages over the island as a whole; this observation is supported by the high frequency of host shifting among avian haemosporidian parasites (6, 13). Although we cannot assess the mechanisms that drive

Fig. 3. Haemosporidian assemblages from selected populations of the bananaquit *C. flaveola*. Lineage OZ21 (*Haemoproteus coatneyi*, a host generalist) occurs in three of five samples; its prevalence is inversely related to the prevalence of the bananaquit specialist lineage, LA07 (*Haemoproteus* sp.). Antigua and Barbuda were broadly connected until 2.5 ka, but both islands have always been separated from Guadeloupe by a deep marine channel. The parasite lineages on Antigua and Barbuda differ from the parasite lineages on Guadeloupe in prevalence, composition, and number of parasite lineages. Parasite assemblages in the Hispaniola (Dominican Republic) samples that are separated by a short time interval have a similar composition, with variation in the prevalence of the dominant lineage, DR03, which is closely related to the bananaquit specialist LA07. The phylogeny represents 132 lineages of haemosporidian parasites from North America, Central America, and the Caribbean. Lineages found infecting the five populations of *C. flaveola* are labeled in the phylogeny (maximum clade credibility tree), showing that lineage composition and replacement on parasite assemblages are phylogenetically uncorrelated.

replacement and host shifting of lineages, the observed shifting of lineage distributions across host species is consistent with the phylogenetic unpredictability of lineage replacement among host populations within an island.

In conclusion, differentiation among haemosporidian assemblages that were presumed to be homogeneous on connected islands until *ca.* 2.5 ka reflects variation in the prevalence of established parasite lineages, and not substitutions, gains, or losses of lineages. In contrast, island-wide lineage replacement drives dissimilarity among haemosporidian assemblages between pairs of islands that have been isolated for longer periods, up to millions of years. Our results suggest that dispersal of parasite lineages among insular host assemblages is limited, and that parasite lineages of particular host species are replaced at random with respect to parasite phylogenetic relationship.

Methods

Study Sites. Field studies were conducted on 17 islands across the West Indies. Formerly connected pairs of islands included the following: (i) Grenada and Carriacou, (ii) Antigua and Barbuda, (iii) St. Kitts and Nevis, (iv) Little Cayman and Cayman Brac, and (v) Eastern Puerto Rico and British Virgin Islands. Each assemblage from these five pairs of formerly connected islands was compared with assemblages on isolated islands, with the exception of Puerto Rico and British Virgin Islands, for which we had no suitable comparison. The isolated islands in each comparison were, respectively, as

follows: (i) St. Vincent, (ii) Guadeloupe, (iii) Montserrat, and (iv) Grand Cayman. Pairwise comparisons between assemblages sampled on the same island, over intervals of up to two decades, were as follows: Dominican Republic (Hispaniola) (2001, 2002, and 2014) and St. Lucia (1991 and 2000). In addition, we sampled haemosporidian assemblages geographically separated within the same island on St. Eustatius, St. Kitts, Nevis, and St. Vincent. Detailed information about study sites and sample collection is provided in *SI Appendix, Table S1*; we present evidence of temporal variation over evolutionary time-scales in Fig. 2 and *SI Appendix, Tables S2–S4*.

Field Methods. We captured birds with mist nets in representative habitats on each island, generally during the late spring and summer months. We took blood samples from captured individuals by brachial venipuncture and stored the samples in Puregene or Longmire's lysis buffer. All samples were collected under Institutional Animal Care and Use protocols approved at the University of Pennsylvania (collections up to 1995) and at the University of Missouri–St. Louis or the National Aviary (after 1995), and under appropriate permits from the governments of the individual islands. Individual birds were released after blood sampling at the site of capture.

Laboratory Methods. We extracted DNA from lysis buffer by isopropanol precipitation preceded by removal of proteins by ammonium acetate precipitation. We used PCR to detect the presence of haemosporidian infections by amplification of a highly conserved 154-bp 16S rRNA-coding sequence of the parasite mitochondrial DNA (14). Samples found to be infected in the first PCR step were further subjected to one or more nested PCR assays that amplify a phylogenetically informative region of the mitochondrial cytochrome *b* gene (*cyt b*) of haemosporidian parasites of the genera *Plasmodium* and *Haemoproteus*. For the first set of nested PCR assays, we used the outer primer pair 3932F and DW4R (18, 19) and the inner primer pair 413F and 926R (20). We also amplified and sequenced regions of the mitochondrial *cyt b* gene from positive samples using a variety of primer pairs and protocols (21–23). Our protocols often fail to recognize mixed infections, meaning that parasite lineage prevalence in host populations may be somewhat underestimated. We distinguished lineages of haemosporidian parasites based on pairwise nucleotide differences between sequences (10).

Phylogenetic Tree Reconstruction. We constructed a phylogenetic tree of 132 haemosporidian lineages from North America, Central America, and the Caribbean region. We used Geneious 9.1.5 to generate an alignment of the 132 *Plasmodium* and *Haemoproteus* sequences, together with 10 *Leucocytozoon* sequences used as outgroup (23). We used BEAST 2.4.2 and its applications (24) to obtain a Bayesian maximum clade credibility tree using an uncorrelated relaxed lognormal clock, a GTR + I + G substitution model, and a Yule speciation prior. Trees were sampled every 5,000th run throughout a Markov chain Monte Carlo chain of 10 million trees and 10% burn-in. Analyses were run using the CIPRES cluster (25). The maximum clade credibility tree was summarized using TreeAnnotator 2.4.2 and visualized using FigTree 1.4.2 (*SI Appendix, Fig. S1*).

Statistical Analyses. For each haemosporidian lineage, we calculated the proportion of infected host individuals, which we refer to as lineage prevalence. Two ratios were calculated using two populations of hosts: (i) captured individuals of six selected focal bird species (host focal assemblages) and (ii) captured individuals of all haemosporidian-infected bird species on an island (island assemblages). Host focal species were the six most well-sampled and widespread avian species for which we had recorded haemosporidian infections: (i) bananaquit, *Coereba flaveola*; (ii) black-faced grassquit, *Tiaris bicolor*; (iii) common ground dove, *Columbina passerina*; (iv) Lesser Antillean bullfinch, *Loxigilla noctis*; (v) pearly-eyed thrasher, *Margarops fuscatus*; and (vi) scaly-breasted thrasher, *Margarops fuscus*. We used these lineage prevalence data to build island × parasite assemblage pairwise comparison matrices. Due to limitations in sample size, we did not include comparisons of contemporary assemblages within islands using host focal assemblage data. We then used the matrices to calculate metrics that describe and partition β diversity to characterize pairwise assemblage comparisons.

We first calculated Chao–Sørensen dissimilarity, which estimates the difference between assemblages on two islands based on lineage prevalence, the number of island-unique lineages, and the number of shared lineages between islands (26). The Chao–Sørensen index is an appropriate choice of a pairwise comparison metric for our data because it is based on frequency data. In our dataset, variation in sample size is directly related to variation in the relative abundance of hosts across islands of the archipelago (14). However, it is important to take into account that the Chao–Sørensen index is blind to variation in the prevalence of lineages present on one island but absent from the other. Chao–Sørensen dissimilarity was calculated by

$$\beta_{\text{Chao-Sørensen}} = 1 - \frac{2UV}{U+V}$$

where U is the sum of the prevalence of all shared lineages (i.e., present on both islands) on one island and V represents the same sum for the other island. When islands of a pair have no shared lineages, dissimilarity equals 1 and similarity equals 0; when islands of a pair have identical composition and prevalence of hemsporidian lineages, the dissimilarity index equals 0 and similarity equals 1.

We then used an additive partitioning method to decompose β diversity into two compartments with different biological meaning: turnover (i.e., replacement) and nestedness (27). This partitioning method is based on the Sørensen dissimilarity index, but also takes into account the proportion of shared lineages among assemblages:

$$\beta_{\text{Sørensen}} = \frac{b+c}{(2a)+b+c}$$

The parameter a represents the number of shared lineages, as above, and the parameters b and c represent, for each island, the number of lineages present on one island but absent from the other. Therefore, $\beta_{\text{Sørensen}}$ combines differences between islands in both the total number of lineages and lineage identity. As the difference between island parasite assemblages increases, $\beta_{\text{Sørensen}}$ increases from 0 to 1. Following Baselga (27), we calculated assemblage turnover as

$$\beta_{\text{Turnover}} = \frac{\min(b,c)}{a+\min(b,c)}$$

Then, we calculated $\beta_{\text{Sørensen}} - \beta_{\text{Turnover}}$ as the nested component of β diversity ($\beta_{\text{Nestedness}}$). As an example of assemblage turnover, bananaquits (*C. flaveola*) from Grenada are infected by the lineages OZ21 and OZ04, whereas on Carriacou, an islet connected to Grenada by a land bridge until ca. 2.5 ka, OZ21 is replaced by lineage LA07. On Barbuda, bananaquit hosts are infected by two hemsporidian lineages, LA07 and OZ21. However, on Antigua, an island connected to Barbuda during the Last Glacial Maximum (LGM), bananaquits are infected solely by OZ21, which makes Antigua a nested subset of Barbuda's hemsporidian assemblage. We used the R package betapart (28) to calculate and partition $\beta_{\text{Sørensen}}$ dissimilarity.

We applied a metric analogous to $\beta_{\text{Sørensen}}$ and its partitions to weight dissimilarity by the phylogenetic distance between lineages among assemblages (29). To calculate phylogenetic β diversity, we used the same parameters a , b , and c described above, but weighted by the total branch length of lineages from the hemsporidian phylogeny we generated. The phylogenetic β diversity of an assemblage (PD) is the sum of the length of all branches from all lineages present in that assemblage. When comparing two assemblages, phylogenetic β diversity represents the variation in composition due to differences between the total PD s of each assemblage (i.e., phylogenetic nestedness) and due to the turnover of lineages that are not related to differences in PD among assemblages (phylogenetic turnover). β_{PhyloSor} and its partitions are calculated by

$$\beta_{\text{PhyloSor}} = \frac{2PD_{\text{total}} + PD_k + PD_j}{PD_k + PD_j}$$

and

$$\beta_{\text{PhyloSor-Turnover}} = \frac{\min(PD_{\text{total}} - PD_k, PD_{\text{total}} - PD_j)}{PD_k + PD_j - PD_{\text{total}} + \min(PD_{\text{total}} - PD_k, PD_{\text{total}} - PD_j)}$$

where PD_k and PD_j are the sum of branch lengths of lineages from

assemblages k and j , respectively, and PD_{total} is the sum of branch lengths from all lineages present in assemblages k and j :

$$\beta_{\text{PhyloSor-PD}} = \beta_{\text{PhyloSor}} - \beta_{\text{PhyloSor-Turnover}}$$

To determine whether assemblages are more evolutionarily related than expected by chance, which likely indicates environmental (including host) filtering, we compared phylogenetic and taxonomic β diversity among pairs of assemblages. Low phylogenetic and high taxonomic β diversity suggests that assemblages share closely related, but not identical, lineages of parasites. In contrast, high phylogenetic and low taxonomic β diversity suggests exclusion of phylogenetically related lineages, potentially the result of parasite competition.

We also calculated the standardized effect size (SES) for β_{PhyloSor} and its partitions, using the formula:

$$SES = \frac{\beta_{\text{obs}} - \text{mean}(\beta_{\text{null}})}{sd(\beta_{\text{null}})}$$

The parameter β_{obs} represents the value of the phylogenetic β diversity index derived from the data, whereas $\text{mean}(\beta_{\text{null}})$ and $sd(\beta_{\text{null}})$ represent the average and the SD of a null distribution of phylogenetic β diversity indices calculated by shuffling labels of lineages in the phylogeny 999 times. Results are displayed in *SI Appendix, Fig. S2*.

Finally, we used linear mixed models to test the null hypothesis that assemblage dissimilarity does not change across the four comparison groups: between islands that have never been connected, between islands connected during the Pleistocene LGM and isolated for ca. 2.5 ka, between samples from the same island separated by short periods of time (i.e., years, decades), and between contemporary samples from the same island. In one set of models, we combined all pairwise comparisons from assemblages of host focal species and treated avian host species identity as a random intercept in the models. Note that comparisons using pooled host focal assemblages did not include contemporary samples from the same island due to sampling limitations. In a second set of models, we used data from the overall island hemsporidian assemblages and treated groups of island comparisons as a random intercept in the models. In both sets of models, by adding a random intercept, we not only acknowledge that there is variation in dissimilarity within each set of comparisons but also address the unbalanced design across groups of pairwise comparisons (*SI Appendix, Table S2*). We ran one model per β diversity metric, requiring 10 models for pooled host focal assemblages and 10 models for island assemblages. All models were weighted by sample size, calculated as the sum of the total number of host individuals captured on each island. Linear mixed models were run using the R package lme4 (30).

ACKNOWLEDGMENTS. Numerous people assisted in the field and laboratory work, including Eldredge Bermingham, Victor Apanius, Gilles Seutin, Irby Lovette, Sylvia Fallon, Bethany Swanson, Julia Gray, Maria Svensson-Coelho, Diana Outlaw, Maria Pil, Victor Penha, Michael Ludlow, and Hannah Madden. None of this work would have been possible without the support and assistance of numerous local government agencies and wildlife officials. Financial and in-kind support for field and laboratory work has been provided over the years by the National Science Foundation, the National Aviary, the National Geographic Society, the Sigma-Xi Foundation Society, the Waitt Foundation, the Falconwood Foundation, the University of Missouri-St. Louis Biology Graduate Student Association, the Whitney R. Harris World Ecology Center at the University of Missouri-St. Louis, and the Curators of the University of Missouri.

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