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Insularity effects on the assemblage of the blood parasite community of the birds from the Gulf of Guinea

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Abstract

Aim—Lower species diversity, increased population densities and ecological niche enlargement are common characteristics of island faunas. However it remains to be determined if they extend to the parasite community. We tested if Haemosporidia parasite pressure varies between islands and the mainland with two different levels of analysis: i) at the host community level, and ii) with paired-species comparisons between islands and the mainland.

Location—Gulf of Guinea, West Africa.

Methods—We used molecular-based methods to identify avian Haemosporidian parasites (*Plasmodium*, *Haemoproteus* and *Leucocytozoon*) to describe their diversity, prevalence, host specificity and their phylogenetic relationships in five islands of the Gulf of Guinea and in nearby mainland areas.

Results—We found reduced Haemosporidia diversity on islands for *Haemoproteus* and *Leucocytozoon*, but not for *Plasmodium*. In addition, lower parasite prevalence on islands was

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SUPPORTING INFORMATION

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

DATA ACCESSIBILITY

Genetic mtDNA data generated for this study are available on GenBank: KT376897-KT376976 and KT595662-KT595669.

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found using a paired-species approach. Although the mean host specificity of the parasite community on islands did not differ from the mainland, we found a very distinct parasite species assemblage on the islands, which was composed of both the most generalist and the most specialist lineages.

Main conclusions—This study supports the hypothesis that parasite pressure is reduced on islands. Colonization is made by generalists with high host switching capacities, with some subsequently evolving into highly specialised parasites. This suggests that 'taxon cycle' dynamics may explain the assemblage of insular parasite communities.

Keywords

avian malaria; *Haemoproteus*; host specificity; island biogeography; *Leucocytozoon*; *Plasmodium*; taxon cycle

INTRODUCTION

Island faunas are often characterized by lower species diversity, ecological niche enlargement, and increased population densities (MacArthur & Wilson, 1967; MacArthur *et al.*, 1972; Buckley & Jetz, 2007). To date, it remains uncertain if and how these patterns described for insular vertebrates apply to their parasite fauna (Illera *et al.*, 2015; Jean *et al.*, 2016). Although island biogeography of parasites and their colonization histories have received increasing attention in recent years (e.g. Cornuault *et al.*, 2012; Sari *et al.*, 2013; Clark & Clegg, 2015), studies thus far have investigated the effects of insularity on parasite diversity and phylogenetic relationships on islands only (Ishtiaq *et al.*, 2012; Carlson *et al.*, 2013; Olsson-Pons *et al.*, 2015; Ricklefs *et al.*, 2016), or have compared islands with the mainland but taking into consideration only one host species (Pérez-Rodríguez *et al.*, 2013; Sari *et al.*, 2013). It is therefore of interest to test if the core predictions of island biogeography theory apply to parasite communities on a broader scale, i.e. by comparing parasite parameters in multiple host species on islands and the mainland.

The first prediction of a depauperate parasite fauna on islands has been confirmed by several studies (Steadman *et al.*, 1990; Fallon *et al.*, 2005; Ricklefs *et al.*, 2011; Spurgin *et al.*, 2012). Nevertheless, few of them have specifically compared island hosts with their counterparts on the mainland, which is important given the geographical and phylogenetic factors that are known to have a strong influence on parasite distributions (e.g. Fallon *et al.*, 2005; Beadell *et al.*, 2006). If islands do exhibit low parasite diversity in relation to the nearby mainland areas it is expected that, on average, parasite species on islands will have broader niches (i.e. broader range of host species) than on the mainland. This would be driven by two distinct processes: i) community sorting: at the time of colonization, generalist parasites from the mainland should be more successful in establishing viable populations by being able to infect multiple and novel host species (taxon cycle; Wilson, 1961; Ricklefs & Bermingham, 2002); ii) island populations will experience less interspecific competition relatively to the species-rich mainland communities, which will open the way for niche enlargement (MacArthur & Wilson, 1967). This decrease of average host specificity will allow for the maintenance of large populations and hence decrease the extinction risk (Pérez-Rodríguez *et al.*, 2013). Finally, to test the prediction of increased parasite density on islands

one can use parasite prevalence (the proportion of individuals infected in a population) as a proxy. Increased density of hosts is thought to arise from a mixture of reduced interspecific competition and release from predation (MacArthur *et al.*, 1972; Buckley & Jetz, 2007). It is less clear which mechanisms may affect parasites, but we can predict an increase of parasite prevalence in two complementary ways: i) by a direct effect, with a lower interspecific parasite competition allowing for higher parasite densities to build up; and ii) indirectly, with increased densities of host populations facilitating parasite transmission (see review in McCallum *et al.*, 2001).

Understanding the characteristics of insular parasites is important, as it should play a major role on the evolutionary dynamics and assembly of communities on islands. First, a release from 'natural enemies' (e.g. pathogens and predators) in island environments is often postulated as one of the key factors promoting demographic and evolutionary change on islands (MacArthur & Wilson, 1967; Ricklefs & Bermingham, 2002; Torchin *et al.*, 2003). Second, parasite pressure is expected to influence the hosts' investment in immunity (Lindström *et al.*, 2004; Matson, 2006; Beadell *et al.*, 2007), and hence environmental variation in pathogen levels should be a main determinant of the evolution of pathogen resistance. If island hosts harbour fewer parasites, they are expected to have evolved reduced defences and, as a consequence, island hosts are often portrayed as being particularly vulnerable to introduced diseases (Wikelski *et al.*, 2004). This is illustrated most notably by the dramatic population declines and extinction of endemic birds in the Hawaiian islands following the introduction of avian malaria and their vectors (Warner, 1968; van Riper *et al.*, 1986; LaPointe *et al.*, 2012).

In this study, we tested the predictions of island biogeography theory in parasites using avian haemosporidian parasites in five Gulf of Guinea islands (Africa) and nearby mainland areas. Birds are commonly infected by three closely related genera (*Plasmodium, Haemoproteus* and *Leucocytozoon*) that are transmitted by the bite of an arthropod host: *Plasmodium* is transmitted by mosquitoes (Culicidae), *Haemoproteus* by midges (Ceratopogonidae) and louse flies (Hippoboscidae), and *Leucocytozoon* by black flies (Simulidae; Valkiūnas, 2005; LaPointe *et al.*, 2012).

Here, we considered two different levels and types of analysis. First, we compared how the parasite pressure differs between islands and the mainland using the whole host community. Second, at a finer scale, we compared island birds with their mainland counterpart (conspecific population or sister/closely-related species). We examined how insularity affects: 1) the diversity of *Plasmodium, Haemoproteus* and *Leucocytozoon* lineages and their phylogenetic relationships, 2) their evolutionary strategy in terms of host specificity (i.e. generalist versus specialist) and 3) their prevalence. According to the predictions of island biogeography theory, we expected lower diversity, lower host specificity of parasites and higher prevalence in island hosts.

MATERIALS AND METHODS

Study area

Sampling was carried out on five islands of the Gulf of Guinea: a land-bridge island (Bioko), three oceanic islands (Príncipe, São Tomé, Annobón) and one islet (Boné), and on the nearby mainland in Cameroon and Gabon (Fig. 1; Appendix S1, Table S1.1). The islands are the offshore part of the 1000 km Cameroon Line of Tertiary to Recent volcanoes, which extends from Annobón to the Mandara Mountains on the Nigeria-Cameroon border (Burke, 2001). Volcanic activity in the continental and oceanic sector has been contemporaneous and more or less continuous since the Cretaceous (Burke, 2001).

The region has an equatorial climate with the year being divided into rainy and dry seasons. The natural habitat type of the Gulf of Guinea islands has been described as rainforest (Exell, 1944; Exell, 1973). The forest is stratified with altitude into lowland rainforest (0–800m), montane forest (800–1400), and mist forest (1400–2500), the latter being absent from Príncipe and Annobón (Exell, 1944). On the islands, most of the lowland forests and some montane forests are replaced by coffee and cocoa plantations, and only south-west and central São Tomé and the South of Príncipe remain covered by relatively undisturbed forest. Today, we can define four types of habitats in order of increasing anthropogenic influence: old-growth rainforest, secondary forest, shade plantation and non-forested habitats (de Lima *et al.*, 2013). We sampled in old-growth rainforest, in shade plantation and in non-forested habitats. Shade plantation refers to agroforestry areas that have crops (cocoa or coffee) growing under the canopy of trees, and the non-forested category includes man-made habitats, such as oil palm monocultures and savannahs (de Lima *et al.*, 2013). In our analyses, we grouped together the habitats “shade plantation” and “non-forested habitat” under the term “plantation” that refers to modified-human habitat in contrast to “forest” which is undisturbed (i.e. old-growth rainforest). On the mainland, we sampled birds in similar types of habitats.

Host species and data collection

Data collection took place from October to April (2002–2014) coinciding with the most important breeding periods both on the islands and the mainland (Appendix S1, Table S1.2). Birds were captured with ECOTONE (Poland) mist-nets, banded with a metal ring, measured and weighted. In addition, a small amount of blood was collected from the brachial vein and stored in ethanol for subsequent molecular analyses.

Sampling sites used for the analysis at the community level were located from sea level to 2400 m and they were either in forest or in plantations (Appendix S1, Table S1.1). For the paired-species analysis, among all the sites, we used samples from sites located only in plantations at the same altitude (below 600 m) on both islands and mainland to control for the habitat and altitude effect.

At the community level, we captured a total of 1396 birds from 13 families and 72 species, targeting both endemic and non-endemic host species, between 2002 and 2014 (Table 1; Appendix S1, Table S1.3). We also gathered additional data from the MalAvi database

(Bensch *et al.*, 2009; Accessed on 05 September 2016) from the same 13 families of three countries bordering the Gulf of Guinea: Cameroon, Gabon and Nigeria (Fig. 2; Table 1).

For the paired-species analysis, we restricted the samples to those collected in 2013 and 2014 (N=580 individuals) from six of the 13 families, for a total of 21 species corresponding to 11 groups of two or three species (Table 2). Species present in both island and mainland locations were paired together; this was the case of *Ploceus cucullatus, Euplectes hordeaceus, Lonchura cucullata* and *Cyanomitra olivacea*. Otherwise we paired species according to their phylogenetic proximity (Table 2), based on molecular phylogenies or on current taxonomy (e.g. *Turdus olivaceofuscus*, Melo *et al.*, 2010). If the closest relative did not occur in the sampling site on the mainland, we used the congeneric species present (e.g. *Estrilda* spp; see also Lobato *et al.*, 2017). In addition, *Ploceus cucullatus* is the closest mainland relative of both the São Tomé endemic *P. grandis* and the Príncipe endemic *P. princeps* (Staffan Andersson, Göteborg University, pers. comm.) and hence, in addition to being paired to a conspecific population present on São Tomé, it was compared to the two island endemics in another paired comparison (pairs 8 and 9; Table 2).

Molecular analyses

Haemosporidian parasites screening—To test for the presence of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* we used the protocol and the cycling profile conditions described in Hellgren *et al.* (2004). In all samples from *Ploceus cucullatus* from Gabon we obtained an unusual band profile. In this species, two bands of very strong intensity of approximate sizes of 480 and 250 bp were systematically obtained when amplifying the *Haemoproteus* and *Plasmodium* fragment. Because modifying cycling conditions did not help, we used the protocol of Drovetski *et al.* (2014), in which three primer pairs are used, to obtain the prevalence of malaria parasites for this species. Because in our comparative approach we paired *P. cucullatus* from Gabon with *P. cucullatus* and *P. grandis* from São Tomé, and *P. princeps* from Príncipe, samples from these species were also screened using the protocol of Drovetski *et al.* (2014) in order to be comparable with *P. cucullatus* from the mainland.

Sequencing and lineage identification—All samples were screened twice in independent PCRs to confirm positive or negative scores. When the two independent PCRs gave different results, a third PCR was performed to confirm positive or negative scores. All PCR reactions included at least a positive sample from previous assays and a negative control. PCR products from confirmed positives were purified for cycle sequencing reactions using ExoSAP-IT (USB Corporation) following the manufacturer's instructions. Bi-directional sequencing was performed with dye-terminator fluorescent labeling in a 3130xl Genetic Analyzer (Applied Biosystems). The sequences were edited and aligned using the program GENEIOUS 7 (Kearse *et al.*, 2012). Haplotypes obtained were compared with sequences available in GenBank and MalAvi (Bensch *et al.*, 2009) databases. Parasite sequences that differed by one or more base pairs were treated as distinct lineages (Bensch *et al.*, 2009; Appendix S2, Table S2.1 and S2.2 for a complete list of lineages and distribution). Multiple infections were observed in a number of samples. Double infections

were resolved when only one double peak was observed, otherwise we gave a score of 'multiple infection' to the sample but we did not obtain the separate lineages (n=106).

Statistical analyses

Statistical analyses were performed for each parasite genus separately, both at the community level and in the paired-species design. To determine whether there were differences in parasite levels between the islands and the mainland at the community level, we used generalized linear mixed models (SAS, 1999) to investigate variation in the following variables: 1) parasite diversity, i.e., number of lineages found per region and per bird family, 2) parasite prevalence, i.e., percentage of infected individuals, and 3) host-specificity. We used and combined data from available online databases and our own sequences to estimate parasite diversity per region (five islands and three mainland regions) and to calculate host specificity indices (Hellgren *et al.*, 2009).

To investigate differences in parasite diversity between the islands and the mainland, we gathered information on the number of lineages of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* found per bird family with i) our sampling in each of the seven regions described above (five islands and two mainland regions; n=145 lineages) and ii) additional information from online databases in three mainland regions (Cameroon, Gabon and Nigeria, n=103; Fig. 2). We used 'insularity' (mainland versus island) and the status of birds (endemic or non-endemic) as fixed factors, and we accounted for host phylogeny by including a random factor 'family' and a random factor 'region' in the models. In addition, estimates of lineage diversity per region were also conducted through rarefaction and extrapolation curves (with 95% confidence intervals) using ESTIMATES 9.1.0 (Colwell *et al.*, 2013; Appendix S2, Fig. S2.1).

For prevalence, we used a binomial distribution (infection = 0 or 1). Patterns of prevalence variation were inferred from our data only because prevalence data could not be extracted from online databases. We included a fixed factor 'insularity' and controlled for several other factors: habitat (forest versus plantation), altitude (continuous variable), year of sampling (2002 or 2014), as well as the random factors 'family' and 'region'.

Host specificity of each parasite lineage from our survey and from the MalAvi database was estimated using the modified version of the host specificity index (Poulin & Mouillot, 2003; Hellgren *et al.*, 2009) that accounts both for the number of hosts species of the parasite and the taxonomic distance among them, and for the variance of the taxonomic distance among host species. First, in our statistical model for the host specificity index (log-transformed), we tested the effect of insularity using the presence/absence of the lineage on islands, on the mainland or on both. We compared the host specificity indices of all lineages found on the islands versus all the lineages detected on the mainland. Then, with our data only, we compared the prevalence of specialist and generalist lineages on islands versus mainland (Fig. S2.2). We designated a lineage as specialist when it infected one, two or three species of the same genus; a lineage infecting two species of two different genera or families was considered as generalist. We used as fixed factors the 'insularity' effect (island versus mainland), as well as other factors (i.e. habitat, altitude, year) and the random effect 'region'.

For the paired-species design, we used the package 'lme4' (Bates *et al.*, 2015) in R (R Core Team, 2015). We tested the insularity effect using generalized linear mixed models on parasite diversity and prevalence but this time with the random effect 'species' nested in 'pair' which was in turn nested in 'family'. Since we formed two pairs including species with different genera (pairs 1 and 5; Table 2), we performed the statistical analyses both including and excluding them (Appendix S2).

Phylogenetic analyses

For the three haemosporidia genera we recovered all sequences available on MalAvi for the Gulf of Guinea. Relationships among lineages were inferred with Bayesian methods, both as implemented in MRBAYES 3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) and in BEAST 1.8.2 (Drummond *et al.*, 2012). We used PARTITIONFINDER 1.1.1 (Lanfear *et al.*, 2012) to determine the best-fit partitioning scheme for the cytochrome *b* dataset (486 bp) and the respective substitution models for each partition, using the Bayesian information criterion (detailed methods in Appendix S3).

RESULTS

Parasite diversity

In this study, we described 81 new Haemosporidian lineages and recorded 64 previously described ones (Appendix S2; Table S2.1 and S2.2) from the Gulf of Guinea but also from other locations of the world. *Plasmodium* was represented by the highest number of lineages on the islands (n=29), followed by *Haemoproteus* and *Leucocytozoon* (17 and 15 respectively; Table 1, Fig. 2). In addition, the number of lineages found exclusively on islands (i.e. endemic lineages) was high, ranging from 65% for *Haemoproteus*, 53% for *Leucocytozoon*, and 41% for *Plasmodium* (Fig. 2; Appendix S2). On the mainland, we found respectively 136, 107 and 109 of *Plasmodium*, *Haemoproteus* and *Leucocytozoon* lineages (Fig. 2), including those retrieved from the online database MalAvi (Table 1, Fig. 2).

Parasite diversity was significantly reduced on the islands for *Haemoproteus* (community level: $P = 0.04$; paired-species design: $P = 0.005$) and *Leucocytozoon* (community level: $P = 0.001$; paired-species design: $P = 0.0003$), but not for *Plasmodium* (community level: $P = 0.34$; paired-species design: $P = 0.42$). These results were confirmed by rarefaction and extrapolation curves (Appendix S2, Fig. S2.1).

Prevalence

With the community approach we failed to detect any insularity effect on the prevalence of the three Haemosporidia genera (*Plasmodium* $F_{1,1081} = 2.85$, $P = 0.091$; *Haemoproteus* $F_{1,1081} = 1.04$, $P = 0.307$; *Leucocytozoon* $F_{1,1327} = 0.02$, $P = 0.885$). However, the paired-species design found a lower prevalence on the islands for all genera (*Plasmodium* $P = 0.011$; *Haemoproteus* $P < 0.0001$; *Leucocytozoon* $P < 0.0001$).

Although insularity had no effect on prevalence at the community level, other factors explained the variation in prevalence, such as altitude (*Leucocytozoon* $F_{1,1327} = 76.81$, $P <$

0.0001; higher prevalence above 300 m) and habitat (*Plasmodium* $F_{1,1081} = 10.54$, $P = 0.001$; *Leucocytozoon* $F_{1,1327} = 5.51$, $P = 0.019$). We found an opposite effect of habitat for these two genera, with a higher *Plasmodium* prevalence in plantation (forest: 15.62%, plantation: 33.08%) and a higher *Leucocytozoon* prevalence in forest (forest: 38.43%, plantation: 23.25%).

Host specificity and phylogenetic relationships

Using the online database MalAvi we calculated the host specificity indices for each lineage recovered in the Gulf of Guinea. Indices ranged from 0 (specialist parasite found in only one host species) to 90.33 (*Plasmodium* sp. SGS1, a generalist found in 106 species) and are given in Appendix S2 (Table S2.1). Overall we did not find an insularity effect on the mean host specificity index for any of the haemosporidian genera (*Plasmodium*: $F_{1,2} = 0.03$, $P = 0.871$; *Haemoproteus*: $F_{1,2} = 0.10$, $P = 0.748$; *Leucocytozoon*: $F_{1,2} = 0.95$, $P = 0.332$; Fig. 3). However, when looking closely at the assemblage of lineages, we found that on the islands, the community of parasites was composed of i) lineages exclusively found on islands (endemic lineages) with a low host specificity index (i.e. specialist parasites) and ii) lineages found at both islands and mainland sites with a high host specificity index (i.e. generalist parasites; Fig. 3; Appendix S2, Tables S2.1 and S2.2); whereas the lineages exclusively found on the mainland had a host specificity index two to ten times higher than on the islands (Fig. 3).

On the islands, we found that generalist lineages were more prevalent than the specialists for the three genera (*Plasmodium*: 15.4% of specialists, 84.6% of generalists; *Haemoproteus*: 46.25% of specialists, 53.75% of generalists; *Leucocytozoon*: 23.7% of specialists, 76.3% of generalists). On the mainland, *Plasmodium* had a similar behaviour (24.1% of specialists, 75.9% of generalists), whereas specialist lineages were more prevalent for both *Haemoproteus* (62.3% of specialists, 37.7% of generalists) and *Leucocytozoon* (78.1% of specialists, 21.9%). These differences were only significant for *Leucocytozoon* ($F_{1,160} = 3.69$, $P = 0.05$; Appendix S2, Fig. S2.2).

Finally, phylogenetic inference showed that most parasite lineages on the islands are the result of independent colonizations (Appendix S3, Fig. S3.1 and S3.2). Many of these went on to differentiate *in situ* leading to the origin of putatively endemic lineages through anagenesis. In only a few cases endemic lineages underwent cladogenesis within the archipelago (e.g. *Haemoproteus* lineages in the white-eyes, Zosteropidae) or even within-islands (e.g. *Plasmodium* lineages in the São Tomé thrush, *Turdus olivaceofuscus*). Whilst absolute divergence dates cannot be estimated due to the uncertainty regarding the rates of evolution of the cytochrome *b* in the three Haemosporidian genera analysed here, the data nevertheless suggest a wide range of ages for endemic lineages.

DISCUSSION

This study provides strong support for the prediction of reduced parasite diversity on islands, although this was not significant for one of the three genera of Haemosporidia analysed here (*Plasmodium*). Parasite prevalence (an indicator of parasite ‘population density’ and infection success) was affected by insularity but in the opposite direction to the density

compensation hypothesis, i.e. it decreased instead of increasing on the islands. We also showed that the parasite community on islands was composed of both very specialized, endemic, parasites and of highly generalist lineages also present on the mainland. The arrival of generalist lineages that, in some cases, evolved *in situ* to become endemic specialists, is a pattern expected from the taxon cycle hypothesis (Wilson, 1961) and was supported by our phylogenetic analyses that confirmed the importance of multiple colonizations for the build-up of the parasite communities of the Gulf of Guinea islands.

Parasite diversity

Estimating true parasite species diversity is greatly affected by sampling effort (Walther *et al.*, 1995). Additional sampling on the islands could reveal additional rare Haemosporidian lineages but we believe that additional sampling on the mainland – a highly biodiverse and poorly explored region – would lead to the discovery of a greater number of lineages than for the islands. Given the large differences found here and considering that the sampling effort was greater on the islands, our main conclusion of overall higher diversity of lineages on the mainland is, if anything, more likely to be strengthened with further sampling. One could also argue that what we considered an endemic lineage could reflect a bias in sampling effort and that these are simply lineages not found on the continent yet. This could be of concern if we relied on our sample collection only. However, we used the large database MalAvi (Bensch *et al.*, 2009) that comprises more than 1800 records in sub-Saharan Africa including 648 described lineages. The use of this database brings a greater confidence in the interpretation of our results.

For *Plasmodium*, we found a relatively high diversity of lineages on islands and this could be due to two non-exclusive phenomena: 1) *Plasmodium* parasites may have a higher colonization rate as indicated by their lower endemicity level and 2) *Plasmodium* lineages may be, in general, more successful colonisers than *Haemoproteus* and *Leucocytozoon* because of their potential higher adaptability and/or a better within-host competitive success (van Rooyen *et al.*, 2013). In relation to *Plasmodium*, the insular *Haemoproteus* and *Leucocytozoon* community was characterised by lower diversity and higher endemism. The co-existence of a high proportion of specialised lineages found exclusively on the islands with a few very generalist lineages, shared with the mainland, could indicate that the genera *Haemoproteus* and *Leucocytozoon* may be in the contracting phase of a taxon cycle (Wilson, 1961) – where diversity decreases as specialised lineages go extinct at a higher rate than the establishment of new colonisers from the mainland.

Prevalence

At the community level, parasite prevalence on islands did not differ from the mainland. However, when using the paired-species comparison, we found that the prevalence of parasite infections significantly decreased on islands. Hence, although we did not find evidence for density compensation in Haemosporidia arising from reduced species diversity (as predicted by the island biogeography theory), this result does further support the hypothesis that parasite pressure is lower on islands. In our study, the generalist lineages were the most prevalent on islands. This generalist trend should lead to a higher probability of co-infections. As generalist parasites are considered poor competitors (Hellgren *et al.*,

2009), an increase of co-infections on islands could play a role in limiting parasite densities and prevalence. Previous studies on the prevalence of blood parasites on islands were inconclusive, with either higher (Illera *et al.*, 2015) or lower prevalence on islands (Pérez-Rodríguez *et al.*, 2013). These conflicting results may arise from the fact that parasite prevalence is also likely to vary in relation to environmental and local factors. For example, the effect of habitat on *Plasmodium* and *Leucocytozoon* prevalence is probably associated with the ecology of their distinct vectors. *Plasmodium* prevalence was higher in disturbed habitats, which is in accordance with several studies showing that habitat disturbance is associated with an increase of *Plasmodium* prevalence (Chasar *et al.*, 2009; Sehgal, 2010). The mosquitoes that transmit *Plasmodium* may favour the open areas created in human-modified habitats (Patz *et al.*, 2000; Yasuoka & Levins, 2007; Vittor *et al.*, 2009). On the contrary, *Simulium* black flies, vectors of *Leucocytozoon*, need lotic microhabitats for laying their eggs and are particularly sensitive to physicochemical characteristics of streams (Stangler *et al.*, 2013). This could contribute to a preference of the vectors for pristine forest where flowing water is not disturbed or polluted (Docile *et al.*, 2015). This could also contribute to explain why *Leucocytozoon* prevalence was found to increase at higher altitudes, as habitat disturbance decreases with altitude (de Lima *et al.*, 2013). Additional research will be necessary to understand the complex web of factors affecting parasite prevalence in general, and on islands in particular. This must necessarily involve comprehensive studies on the distribution and ecology of the vectors (Bataille *et al.*, 2012; Santiago-Alarcon *et al.*, 2012). To date, almost nothing is known about vectors in the Gulf of Guinea islands (Ribeiro *et al.*, 1998, Mustapha *et al.*, 2004, 2006); further study and sampling of mosquitoes, midges and black flies is therefore the next step in our ongoing research in the area.

Host specificity and parasite community assembly on the islands

Using all the data available on MalAvi database, we found a similar average host-specificity on the mainland compared to the islands. A closer look at the distribution of generalists/specialists revealed an interesting pattern. Lineages present both on islands and on the mainland, i.e., the successful colonisers, were by far the most generalist lineages, with a host specificity index mean at least five times higher than the others. On the islands, the parasite community was composed of these generalist lineages and of lineages restricted to the islands with very narrow niches. On islands, the generalist lineages were the most prevalent. This suggests that, as predicted by island biogeography theory (MacArthur & Wilson, 1967), a broad ecological niche would be selected as a strategy that decreases the chances of extinction – in this case of parasites colonizing a new area where potential host populations are small (Beadell *et al.*, 2006). The same pattern was also found for the Haemosporidian parasites of a songbird in the Madeira and the Canary islands (Pérez-Rodríguez *et al.*, 2013).

Phylogenetic analyses showed that the current parasite diversity on the islands derives from multiple colonizations from the mainland followed, in many instances, by *in situ* speciation. Inter-island dispersal was negligible. These results parallel those found across distinct taxonomic groups showing that the Gulf of Guinea is a major centre of endemism where the biota of each island has evolved mostly in isolation from those of the other islands (Jones, 1994; Jones & Tye, 2006). The only case of inter-island dispersal followed by diversification

(archipelago radiation) occurred in the lineages infecting the Zosteropidae clade, whose species in the Gulf of Guinea oceanic islands constitute one of the few examples of archipelago radiation in birds (Melo *et al.*, 2011). This suggests that, in this case, the diversification pattern of the parasite lineages was driven by the dispersal history of the hosts rather than by the dispersal of vectors.

The phylogenetic and host specificity data suggest that the assembly of parasite communities in the Gulf of Guinea islands matches the taxon cycle hypothesis (Wilson, 1961): i) we found that generalist parasite lineages were the most successful island colonisers, and were the most prevalent lineages in the insular bird community; ii) with time, divergence from the mainland relatives accumulates and leads to the evolution of island endemics which are characterised by an increase in host specificity, becoming very narrow specialists, which we found in our system; iii) specialists will be more prone to extinction and will be replaced by new arrivals of generalists. This cyclic pattern of species turnover is described for macro-fauna, but its causes remain poorly understood. The possibility that the taxon cycle may extend to parasites is interesting given that parasites, through the evolutionary arms race with their hosts, have been proposed as one of the agents underlying macro-faunal taxon cycles (Ricklefs & Bermingham, 1999, 2002; Ricklefs *et al.*, 2016).

Do island hosts experience a more benign parasite environment?

Our results, for Haemosporidian parasites from the Gulf of Guinea, support the hypothesis that parasite pressure is lower on islands. We found that islands had generally lower parasite diversity and either similar or reduced prevalence. As a result, in this region, island hosts face reduced diversity of infections and at least some of the host species on islands are less likely to become infected. Additionally, given low host diversity on the islands, it can also be argued that single-host parasites on islands have evolved to become less virulent, decreasing the risk of extinction of the few hosts that allow them to complete their life cycle. Studies on the pathogenicity of the different lineages of the island parasites studied here, as well as additional studies on other parasite groups and island systems, are needed to establish the generality of the patterns revealed in the present study. A reduced parasite pressure is thought to have direct consequences in terms of both health condition and immunological trade-offs of hosts. Understanding the effects of reduced parasite pressure on host life-history strategies and immunity is therefore essential to understand patterns of adaptation on islands as well as to attempt to mitigate the impact of recently introduced pathogens on the endemic communities of oceanic islands.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Biographies

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Figure 1.

Map of the Gulf of Guinea, in central-west Africa, illustrating the seven regions sampled within the five islands and the mainland sampling areas (located in Cameroon and Gabon). White dots represent sampling sites on the mainland and the numbers of sampled individuals are given for each region.

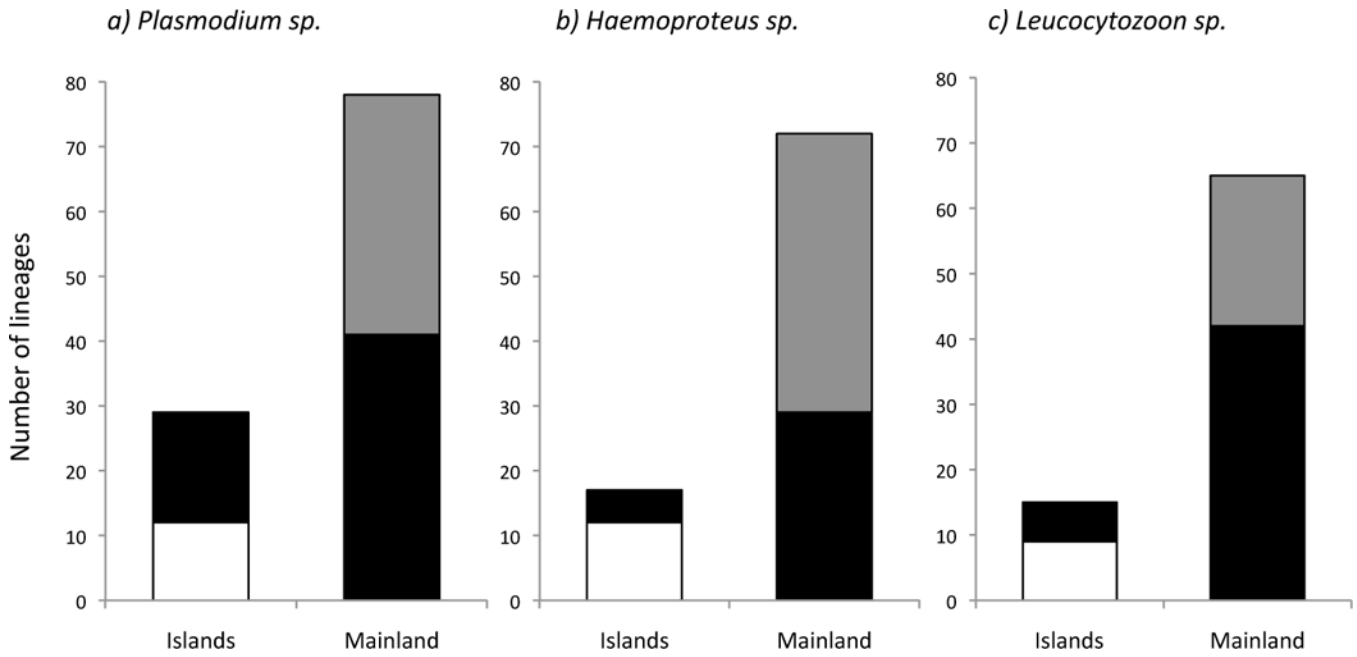

Figure 2.

Number of parasite lineages found on birds from the Gulf of Guinea for a) *Plasmodium sp.*, b) *Haemoproteus sp.*, and c) *Leucocytozoon sp.* Islands: numbers of lineages found on the islands, with the white part representing lineages found exclusively on the islands; Mainland: number of lineages found on the mainland, with the grey part representing lineages from the mainland retrieved from the MalAvi database.

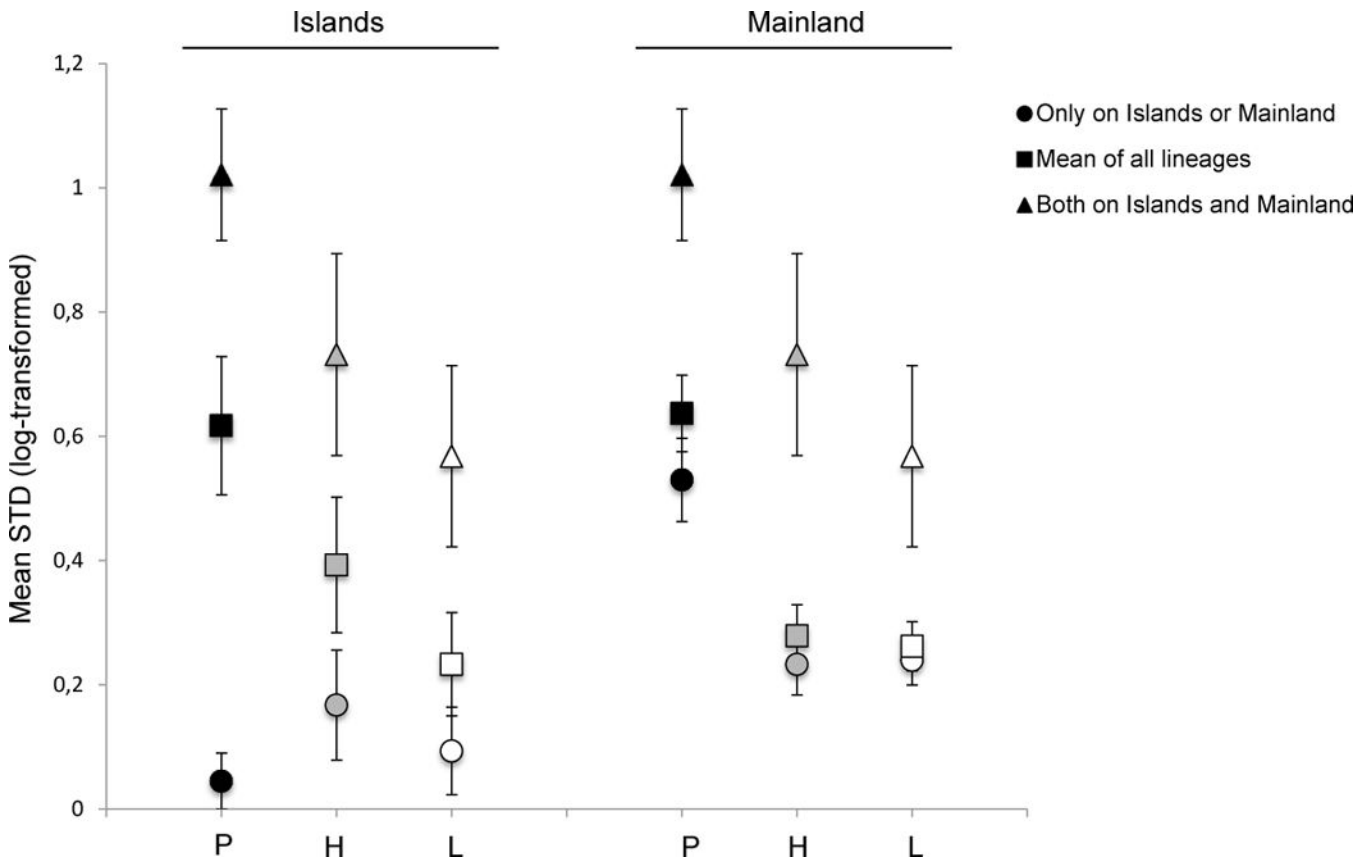

Figure 3.

Host specificity across the three Haemosporida genera found on birds on the Gulf of Guinea islands and mainland. Host specificity estimated with Poulin & Mouillot's (2005) STD* index (mean STD log transformed ± SE). P=*Plasmodium*, H=*Haemoproteus*, L=*Leucocytozoon*. Circles: lineages restricted either to islands or to the mainland; triangles: lineages found simultaneously on islands and the mainland; squares: all lineages. High values represent generalist lineages, and low values represent specialist lineages.

Table 1

Blood parasite diversity and prevalence in birds from the Gulf of Guinea, West Africa. Numbers of lineages of each parasite genera and the prevalence in percentage are given for each of the sampled regions, as well as the additional numbers of lineages gathered online from three mainland regions (#).

			Plasmodium		***Haemoproteus***		***Leucocytozoon***	
		***N* individuals**	***N* lineages**	**Prevalence (%)**	***N* lineages**	**Prevalence (%)**	***N* lineages**	**Prevalence (%)**
Islands	Annobón	12	0	0	1	30	1	25
	Bioko	65	5	39.47	3	18.42	4	49.23
	Boné	51	0	0	0	0	0	0
	Príncipe	321	12	34.68	4	4.43	8	9.87
	São Tomé	549	18	18.68	10	16.09	6	29.12
	Total	*998*	*29*	*24*	*17*	*11.1*	*15*	*22.8*
Mainland	Cameroon	70	8	22.95	7	26.23	7	17.14
	Gabon	328	30	45.68	22	20.68	35	37.19
	Total	*398*	*35*	*42.1*	*28*	*21.5*	*42*	*34.2*
	Cameroon (#)		24		10		1	
	Gabon (#)		12		19		0	
	Nigeria (#)		27		21		24	

Table 2

Blood parasite diversity and prevalence in the Gulf of Guinea across the bird species used in a paired-species comparison between islands and the mainland. *Nind*: number of individuals tested; *N*: number of parasite lineages. *Ploceus cucullatus* from Gabon was used in two paired comparisons (pairs 8 and 9; see text).

					Plasmodium		***Haemoproteus***		***Leucocytozoon***	
Pair	**Family**	**Region**	**Species**	***Nind***	***N***	**Prevalence**	***N***	**Prevalence**	***N***	**Prevalence**
1	*Columbidae*	Gabon	*Turtur afer*	10	0	0 (0/10)	1	10.00 (1/10)	0	10.00 (1/10)
1		Príncipe	*Aplopelia larvata*	13	0	0 (0/12)	1	8.33 (1/12)	0	0 (0/13)
1		São Tomé	*Columba malherbii*	8	0	0 (0/6)	2	33.33 (2/6)	0	37.50 (3/8)
2	*Estrildidae*	Gabon	*Estrilda melpoda*	14	1	7.14 (1/14)	2	92.85 (13/14)	0	78.57 (11/14)
2		Príncipe	*Estrilda astrild*	17	0	0 (0/17)	0	0 (0/17)	0	0 (0/17)
2		São Tomé	*Estrilda astrild*	38	0	0 (0/38)	0	0 (0/38)	1	2.63 (1/38)
3	*Estrildidae*	Gabon	*Lonchura cucullata*	19	1	5.26 (1/19)	2	68.42 (13/19)	0	0 (0/19)
3		Príncipe	*Lonchura cucullata*	19	0	0 (0/19)	1	5.26 (1/19)	0	0 (0/19)
4	*Fringillidae*	Gabon	*Crithagra capistrata*	21	2	28.57 (6/21)	0	0 (0/21)	1	14.28 (3/21)
4		São Tomé	*Crithagra mozambica*	14	3	50.00 (7/14)	0	0 (0/14)	0	7.14 (1/14)
4		São Tomé	*Crithagra rufobrunnea*	21	2	9.52(2/21)	1	75.00 (15/20)	2	66.66 (14/21)
5	*Nectariniidae*	Gabon	*Cyanomitra verticalis*	15	7	53.33(8/15)	0	0 (0/15)	4	26.66 (4/15)
5		Príncipe	*Nectarinia hartlaubii*	10	3	100 (10/10)	0	0 (0/10)	2	80.00 (8/10)
5		São Tomé	*Nectarinia newtonii*	29	6	24.14 (7/29)	1	3.45 (1/29)	2	20.69 (6/29)
6	*Nectariniidae*	Gabon	*Cyanomitra olivacea*	12	4	66.66 (6/9)	2	25.00 (2/8)	5	58.33 (7/12)
6		Príncipe	*Cyanomitra olivacea*	17	5	64.70 (11/17)	0	0 (0/17)	0	17.64 (3/17)
7	*Ploceidae*	Gabon	*Euplectes hordeaceus*	27	5	59.26 (16/27)	2	11.11(3/27)	6	51.85 (14/27)
7		São Tomé	*Euplectes hordeaceus*	23	1	4.35 (1/23)	0	0 (0/23)	0	0 (0/23)
8	*Ploceidae*	Gabon	*Ploceus cucullatus*	46	4	58.53(24/41)	2	11.63 (5/43)	3	57.14 (24/42)
8		São Tomé	*Ploceus cucullatus*	33	4	70.00 (21/30)	0	0 (0/30)	0	0 (0/30)
9	*Ploceidae*	Gabon	*Ploceus cucullatus*	46	4	58.53(24/41)	2	11.63(5/43)	3	57.14(24/42)

Pair	Family	Region	Species	*Nind*	*Plasmodium*		*Haemoproteus*		*Leucocytozoon*	
					N	Prevalence	*N*	Prevalence	*N*	Prevalence
9		Príncipe	*Ploceus princeps*	53	8	52.08(25/48)	0	0 (0/48)	0	0 (0/48)
9		São Tomé	*Ploceus grandis*	16	1	6.25(1/16)	0	0 (0/16)	2	43.75 (7/16)
10	*Ploceidae*	Gabon	*Ploceus nigricollis*	30	3	51.72 (15/29)	3	27.58 (8/29)	11	73.33 (22/30)
10		São Tomé	*Ploceus sanctithomae*	18	3	22.22 (4/18)	0	0 (0/18)	4	77.77 (14/18)
11	*Turdidae*	Gabon	*Turdus pelios*	24	4	95.65 (22/23)	0	0 (0/23)	1	66.66 (16/24)
11		São Tomé	*Turdus olivaceofuscus*	33	4	45.45 (15/33)	0	0 (0/33)	1	18.18 (6/33)