Global Ecology and Biogeography, (Global Ecol. Biogeogr.) (2013) ••, ••–••

Global Ecology and Biogeography
A Journal of Macroecology

RESEARCH PAPER

Evolution of parasite island syndromes without long-term host population isolation: parasite dynamics in Macaronesian blackcaps *Sylvia atricapilla*

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ABSTRACT

Aim The study of parasite biogeography on islands is important for our understanding of both the processes involved in the evolution of parasite assemblages worldwide and the ecology and conservation of insular communities. By studying the haemosporidian blood parasites of a bird that has recently colonized a number of oceanic islands, we were able to test hypotheses relating to the processes of parasite colonization and community assembly prior to the permanent isolation of host species on islands.

Location The Atlantic Ocean archipelagos of Madeira and the Canary Islands.

Methods We used cytochrome *b* DNA sequences to determine the prevalence and richness of parasites of the genera *Haemoproteus*, *Plasmodium* and *Leucocytozoon* in blackcaps, *Sylvia atricapilla*, a widespread passerine that colonized these archipelagos during the Last Glacial Maximum. We compared insular blackcap parasite assemblages with those observed in 37 blackcap populations sampled on mainland Europe.

Results The insular parasite assemblage was impoverished, containing *c.* 10% of the parasites found on the continent. None of the parasites observed on the islands were blackcap specific. Some of the observed parasites appear to have switched from blackcaps to other Macaronesian host species, whereas others were of Afrotropical origin and were acquired after blackcaps colonized the islands. The prevalence of parasites in the island populations of blackcaps was lower than in mainland blackcap populations and parasite richness decreased with increasing island distance to the continent.

Main conclusions Macaronesian blackcaps do not face the strong parasite load encountered by their mainland counterparts despite the fact that blackcap migration from the continent may directly transport mainland blackcap parasites to the islands. This supports the idea that normal mainland host–parasite associations are compromised on islands and that parasite island syndromes (low richness, frequent host-switching and reduced specialization) evolve even before insular host populations become completely isolated from their mainland counterparts.

Keywords

Avian haemosporidians, blackcap *Sylvia atricapilla*, Canary Islands, host specificity, island colonization, island syndromes, Madeira, parasite biogeography.

DOI: 10.1111/geb.12084
http://wileyonlinelibrary.com/journal/geb

INTRODUCTION

Oceanic islands, those that have never been linked to a continent, have been the focus of biogeographical research because of the opportunities they offer to understand the processes involved in species' range expansion and the evolutionary consequences of population isolation (Whittaker & Fernández-Palacios, 2007). Although parasite biogeography is still an emerging field, a number of studies have analysed the patterns of island colonization (Ishtiaq *et al.*, 2010; Cornuault *et al.*, 2012), host–parasite co-evolution across different islands (Fallon *et al.*, 2003) or the development of island syndromes by parasites (changes in life history, behaviour or physiology after island colonization; Nieberding *et al.*, 2006). The island biogeography of parasites is also important for understanding the risk and patterns of disease introduction into new areas. Various studies have addressed what enables a parasite to be a successful invader (Ewen *et al.*, 2012), or instead why some are lost during host range expansion (MacLeod *et al.*, 2010). The implications of this research are many; for instance, the introduction of exotic parasites offers dramatic examples of parasite-driven extinction in insular faunas (Wikelski *et al.*, 2004; Atkinson & LaPointe, 2009).

So far, the study of parasites on islands has mainly focused either on isolated host species, usually insular endemics (Fallon *et al.*, 2005; Illera *et al.*, 2008; Valkiūnas *et al.*, 2010; Howe *et al.*, 2012), or on the arrival of novel parasites to naïve insular faunas because of anthropogenic effects (Atkinson & LaPointe, 2009; Ewen *et al.*, 2012). Although undeniably relevant to our understanding of the evolution of parasitism on islands, these studies involve either too long, or too short, time-scales to capture the natural processes involved in the assembly of insular parasite communities. Insular endemics are no longer affected by parasites arriving with conspecific hosts from the continent, consequently the host–parasite interactions on such islands are already independent from the interactions maintained before island colonization. Meanwhile, anthropogenic parasite introductions may provide valuable information on the earliest stages of parasite colonization but are too recent to allow an assessment of the chances of a parasite's long-term establishment on the insular fauna. An important, yet infrequently used comparison in studies of insular parasites is between mainland and island parasite assemblages of host species that have recently (evolutionary speaking) colonized oceanic islands and still remain linked to the original continental populations by migration. Such comparisons may allow us to test whether the evolution of the typical characteristics of insular parasite assemblages (i.e. parasite island syndromes which typically involve low richness, frequent host-switching and reduced host specialization) is only possible after long periods of isolation on islands, or if they can evolve in the face of sustained contact between insular and mainland host populations.

Avian haemosporidians (order Haemosporida, phylum Apicomplexa) are blood parasites transmitted by the bite of several families of blood-sucking dipteran vectors. They are a diverse group of parasites that infect most bird species (Valkiūnas, 2005). The development of polymerase chain reaction (PCR)-based parasite detection methods has greatly facilitated the analysis of haemosporidians in ecological and evolutionary studies (reviewed in LaPointe *et al.*, 2012), and consequently they have become a preferred model in island parasite biogeography (Fallon *et al.*, 2003; Beadell *et al.*, 2007; Ishtiaq *et al.*, 2010; Ricklefs *et al.*, 2011; Cornuault *et al.*, 2012).

We studied the haemosporidian parasites of blackcaps (*Sylvia atricapilla*) on the Macaronesian archipelagos of Madeira and the Canary Islands. Macaronesia is the collective name of four Atlantic archipelagos (Azores, Madeira, Canary Islands and Cape Verde) of independent volcanic origin but shared biotic features. Whether geographically isolated (Azores), or near the Saharan African coast, their biotas show clear links with the Mediterranean region (Whittaker & Fernández-Palacios, 2007; Fernández-Palacios *et al.*, 2011; Illera *et al.*, 2012). Until recently these islands had been largely ignored by evolutionary biologists in comparison with the attention given to other archipelagos such as Hawaii or Galapagos (Fernández-Palacios *et al.*, 2011; Illera *et al.*, 2012). Consequently, their avian haemosporidian communities still remain relatively poorly studied (Foronda *et al.*, 2004; Hille *et al.*, 2007; Illera *et al.*, 2008; Alcaide *et al.*, 2010; Hellgren *et al.*, 2011; Spurgin *et al.*, 2012). This paucity of research does not reflect the great interest shown to the Macaronesian avifauna that includes both locally evolved ancient endemics and recent colonizer species with broader geographical ranges outside the islands (Illera *et al.*, 2012) and provides an excellent range of different host models for the analysis of parasitism on oceanic islands.

Blackcaps are recent colonizers of the Macaronesian islands. Their arrival on these islands has been broadly dated between 4000 to 40,000 yr BP (Pérez-Tris *et al.*, 2004; Dietzen *et al.*, 2008) and most likely took place during the last 13,000 years through several independent colonization events by migrants from continental Europe (Pérez-Tris *et al.*, 2007; Dietzen *et al.*, 2008). Blackcap populations are resident in Madeira and all of the Canary Islands except the two easternmost ones, but coexist with a regular, though poorly studied, annual influx of migrating and overwintering European blackcaps, which is greater the closer the island is to the continent (Shirihai *et al.*, 2001). Although blackcaps harbour an exceptionally diverse community of avian haemosporidians (Pérez-Tris *et al.*, 2007; Križanauskiene *et al.*, 2010), the only study that has so far analysed blackcap parasite diversity within Macaronesia (on São Miguel Island, Azores) found no infected birds among the 74 examined (Hellgren *et al.*, 2011).

We studied haemosporidian infections of blackcaps on the Canary Islands, Madeira and the Iberian Peninsula to test four predictions derived from current hypotheses on the factors promoting parasite arrival, establishment and loss associated to island colonization:

1. *Parasites that are common on the mainland are more likely to occur on islands.* Because the number of individuals involved in a colonization event is usually small (Whittaker & Fernández-Palacios, 2007), we predict that those parasites that are common in mainland blackcaps are most likely to be transported to islands with colonizing hosts (Ewen *et al.*, 2012).

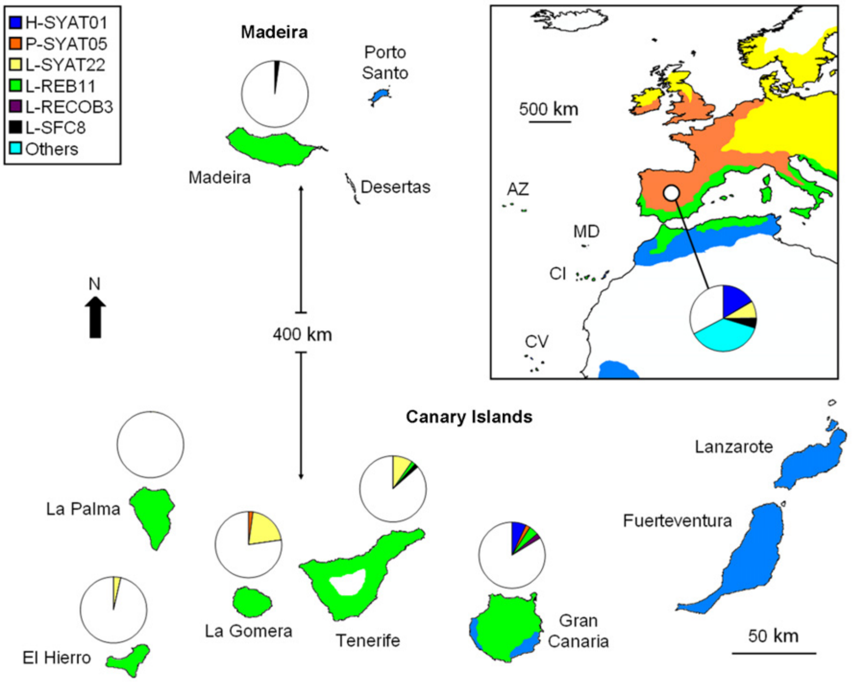

Figure 1 Blackcap distribution on the Macaronesian archipelagos (main map) and across the species' western Palaearctic range (inset map; modified from Shirihai *et al.*, 2001). Population status is shown in different colours (yellow: summer visitor; orange: present year-round, breeding populations migratory; green: present year-round, breeding populations sedentary; blue: winter visitor). The geographic location of the four Macaronesian archipelagos is also shown on the inset map (AZ: Azores Islands, MD: Madeira Islands, CI: Canary Islands, CV: Cape Verde Islands). The pie charts represent the prevalence of the six insular parasite haplotypes (represented by different colours; white areas represent the percentage of uninfected birds) in each of the six sampled islands (data of 2007) and in the Iberian Populations ('others' are those not found on islands – Table S1 –, which have been grouped). Parasite genera are distinguished by the initial of haplotype names (H: *Haemoproteus*, P: *Plasmodium*, L: *Leucocytozoon*).

2. *Generalist parasites will be more likely to occur on islands than specialists.* A scarcity of preferred hosts, particularly at the beginning of the colonization process, should be a handicap for specialist parasites that cannot infect alternative host species (MacLeod *et al.*, 2010; Ewen *et al.*, 2012). Therefore, we expect parasites that can infect both blackcaps and other host species to be more frequent on the islands than blackcap-specific parasites.
3. *The parasite diversity of a specific host should be positively correlated with that hosts' population size on the islands.* If any blackcap-specific parasites made it to the islands they would depend on the availability of blackcaps to thrive. Therefore, we expect the diversity of such parasites to be highest on the islands most populated with blackcaps and lowest on those that are least populated (Altizer *et al.*, 2007).
4. *The parasite diversity on islands will be positively correlated with their proximity to the continent.* The number of migratory continental blackcaps that arrive on an island increases with island proximity to the African coast, thus increasing the chances of parasites being locally established, either because of repeated historical colonization events from the continent, or because of contemporary cross infection from visiting continental hosts. Consequently, parasite assemblages should be structured according to the expectations of island biogeography theory (MacArthur & Wilson, 1967; Fallon *et al.*, 2005).

METHODS

Field sampling

During the blackcap breeding seasons of 2003, 2004 and 2007 (May—July), a total of 380 blackcaps were mist-netted throughout the species' range on the Canary Islands and Madeira, although only in 2007 were all six studied islands sampled (Fig. 1, Table 1). Due to the patchy distribution of blackcaps, individuals were captured at 10–23 sites on each island, depending on island size. Blackcaps were individualized with a metal ring and sexed and aged as juveniles (hatched during the sampling year) or adults (older than 1 year) based on plumage characteristics. A blood sample was collected by venipuncture and stored in absolute ethanol at ambient temperature during fieldwork and then refrigerated until analysed. All birds were released at the site of capture.

Molecular analyses

Genomic DNA was extracted from blood following a standard ammonium acetate protocol. DNA was diluted to a working concentration of 25 ng/μl. In order to check sample quality for PCR, all samples were tested using a bird sexing protocol

Table 1 Sample size per year and parasite screening results. GenBank accession numbers of parasite haplotypes are given between parentheses.

			Parasite haplotypes					
Island	Year	*n*	H-SYAT01 (AY831750)	L-SYAT22 (DQ847236)	L-REB11 (DQ847223)	L-RECOB3 (DQ847221)	L-SFC8 (DQ847234)	P-SYAT05 (DQ847271)
Madeira	2007	43	0	0	0	0	1	0
Gran Canaria	2003	21	0	0	0	0	0	0
	2007	43	3	0	2	1	0	1
Tenerife	2003	18	0	0	0	0	0	0
	2007	51	0	5	1	0	1	0
La Gomera	2004	16	0	0	1	0	0	0
	2007	44	0	9	0	0	0	1
La Palma	2004	23	0	0	0	0	0	0
	2007	43	0	0	0	0	0	0
El Hierro	2003	50	0	0	0	0	0	0
	2007	28	0	1	0	0	0	0

(Griffiths *et al*., 1998), which also provided the gender of juvenile birds that could not be sexed in the field. Samples were screened for *Plasmodium*, *Haemoproteus* and *Leucocytozoon* infections following a nested PCR protocol to amplify 479 bp of the parasite's cytochrome *b* (Hellgren *et al*., 2004). Both positive and negative controls were included in every PCR to check for PCR performance and possible contamination. PCR results were visualized in 2% agarose gels stained with GelRed™ (Biotium, Hayward, CA, USA) under UV light. Negative samples were rerun to check for false negatives; neither false positives (contamination) nor false negatives were detected. All PCR products were sequenced from both ends on an ABI Prism 3730 capillary robot (Applied Biosystems, Paisley, UK). Sequences were edited manually using BioEdit 7.0.5.3 (Hall, 1999) and distinct cytochrome *b* haplotypes – defined by a difference of at least one base in the 479 bp DNA sequence – were identified using BLAST local alignment algorithms on GenBank and MalAvi (Bensch *et al*., 2009) sequence repositories.

Statistical analyses

We used simple regression to test for correlations between the number of parasite haplotypes per island and (1) island distance to mainland (as a correlate of colonization impediment) or (2) size of the blackcap's range on each island (as a surrogate of host population size). We conducted these analyses using only the Canary Islands, excluding Madeira as belonging to a different archipelago, which was independently colonized by blackcaps (Pérez-Tris *et al*., 2004) and thus disconnected from any stepping-stone pattern of colonization of the Canary Islands. We deliberately avoided simultaneously testing for distance and range size effects because of small sample size ($n = 5$ islands). We used the number of Universal Transverse Mercator squares (10 km^2) with confirmed breeding blackcaps on each island (Carbonell, 2003; Equipa Atlas, 2008) as a measure of the blackcap's range on each island. All variables were log-transformed to increase normality of residuals. Because of the small sample size, we also performed Spearman rank correlations to substantiate our conclusions using two different approaches.

We compared parasite diversity found in the island blackcaps with mainland blackcaps using a sample of 882 birds from 37 breeding populations scattered throughout the Iberian Peninsula (see Table S1 in Supporting Information). Iberian blackcap populations accurately represent the parasite assemblages typical for the species in continental Europe (Pérez-Tris & Bensch, 2005; Križanauskiene *et al*., 2010). To control for different sampling effort between island and mainland, we constructed curves of cumulative parasite richness using the software EstimateS 7.5.1 (Colwell, 1999). From these curves we obtained the average number of parasites found after scoring 25 infections (R_{25}), which we used as a comparable estimate of parasite richness between islands and mainland. We derived average curves and R_{25} values from 10,000 richness curves constructed by randomly selecting the order in which individual infections were scored.

We tested whether island and mainland blackcap's parasite communities (as opposed to prevalence) differed significantly. To this end, we compared the parasite observed community on islands with the community expected, under the assumption that the occurrence of a given parasite in a sample of insular blackcaps equals its probability of occurrence in the continent regardless of variation in overall parasite prevalence. To generate a null distribution we simulated a population of 100,000 infected blackcaps, in which each parasite haplotype occurred with the same frequency as observed across all Iberian blackcap populations. This is a conservative approach, because it includes a number of locations where any given parasite was absent, a circumstance that lowers expected probabilities of parasite occurrence. From the simulated population, we randomly selected 10,000 groups of 27 parasite infections (equalling the number of infections retrieved from insular blackcaps; see Results) and measured the prevalence of each parasite in each random trial. We used these

Table 2 Prevalence of the identified insular parasites in island and mainland blackcap populations. For each parasite haplotype, prevalence (mean ± SD) has been estimated from 1000 bootstrap replicates of blackcaps sampled in populations where the corresponding parasite occurred (the number of re-sampled individuals in each case is shown in brackets). Estimates for L-SFC8 changed slightly depending on whether Madeira was included (all islands) or excluded (Canary Islands).

Parasite haplotype	All islands	Canary Islands	Iberian Peninsula
H-SYAT01	6.58 ± 0.23 (43)	6.58 ± 0.23 (43)	17.42 ± 0.08 (755)
L-SYAT22	12.06 ± 0.18 (123)	12.06 ± 0.18 (123)	16.97 ± 0.12 (397)
L-REB11	3.08 ± 0.11 (94)	3.08 ± 0.11 (94)	0
L-RECOB3	2.25 ± 0.14 (43)	2.25 ± 0.14 (43)	0
L-SFC8	2.09 ± 0.09 (94)	1.95 ± 0.12 (51)	9.36 ± 0.09 (431)
P-SYAT05	2.30 ± 0.10 (87)	2.30 ± 0.10 (87)	4.07 ± 0.13 (96)

simulations to estimate the probability of finding particular parasite haplotypes on islands by random sampling of the diversity of mainland blackcap parasites.

We also tested whether parasite haplotypes shared between island and mainland blackcap populations had similar prevalence in both geographical scenarios. We first estimated prevalence of each parasite haplotype on islands and Iberian localities where the parasite was found (i.e. where we were certain the parasite thrives). Given that the prevalence of specific parasites varies among blackcap populations, we computed bootstrap estimates of prevalence for each parasite by resampling all screened blackcaps from the populations in which that specific parasite had been observed. From each sample, we obtained 1000 random samples of the same size (with repetition), which were used to compute the prevalence of each parasite. These estimates where then used to test whether the prevalence of the parasites that were found in insular and Iberian blackcaps was positively correlated between islands and mainland. We also assessed whether the prevalence of these parasites differed overall between islands and mainland, using repeated measures analysis of variance (ANOVA) with parasite haplotype as a within-subject effect.

RESULTS

We found six different parasite haplotypes in blackcaps from Madeira and the Canary Islands (Fig. 1, Table 1). The insular parasite assemblage was poor compared with that found in Iberian blackcap populations (24 haplotypes in total; see Table S1). Insular parasites comprised four haplotypes commonly observed among mainland blackcaps, as well as two haplotypes not previously found infecting blackcaps. Although 54.2% of the parasites found in the Iberian Peninsula were blackcap specific (Table S1), none of these host specialists were present among the six insular parasites. The known host and geographic ranges of these parasites, according to the MalAvi database (Bensch *et al.*, 2009), are provided in the online appendix.

Twenty-seven out of 380 blackcaps were found to be infected on the islands (Fig 1, Table 1). The observed average parasite prevalence (7.1% overall, 10.3% using only the 2007 data) was very low compared with that observed in mainland populations (mean ± standard error = 65.3 ± 4.61%, $n = 37$ Iberian localities). Parasite prevalence varied greatly among years, with no infections found in 2003, one observed in 2004, and 26 in 2007 (Table 1). Overall parasite prevalence also varied significantly among islands (chi-square test with data from 2007: $\chi^2_{(5)} = 18.91$, $P = 0.002$; the results of this test remained significant using birds from all years or excluding Madeira), ranging from 0% in La Palma to 22.7% in La Gomera in 2007.

All parasites shared between insular and continental blackcaps had lower prevalence in the island populations (Table 2), with differences for a given parasite between 29 and 79% less than the prevalence of that same parasite observed on the Iberian Peninsula (repeated measures ANOVA: $F_{1,3} = 11.16$, $P = 0.044$; excluding Madeira: $F_{1,3} = 10.37$, $P = 0.049$). Nevertheless, the prevalence estimates of parasites that were found on island blackcaps were still positively correlated with those in the Iberian populations (Pearson's $r = 0.80$; excluding Madeira: $r = 0.79$; Table 2). Although these correlations were not significant due to small sample sizes ($P = 0.19$ and 0.21, respectively, $n = 4$ haplotypes; Spearman rank correlations, both $r = 0.60$, $P = 0.40$), they support our hypothesis that relative differences in prevalence among blackcap parasites on islands were similar to those observed on the continent.

The impoverishment of the insular parasite assemblage compared with that on mainland Europe was further supported by the difference observed in haplotype richness when controlling for sampling effort (mean ± standard deviation: islands: $R_{25} = 5.93 \pm 0.48$; mainland: $R_{25} = 10.95 \pm 2.33$; $t = 284.26$, $n = 10{,}000$ curves, $P < 0.001$; Fig. 2). The random loss of rare parasites alone did not fully explain the impoverished island parasite assemblages, as shown by the fact that three *Haemoproteus* haplotypes (H-SYAT02, H-SYAT03 and H-SYAT14) that were extremely likely to be present on the islands (given that they were very common on the mainland) were not found (Fig. 3). Many other haplotypes reached high prevalence in the Iberian Peninsula but were not found on islands, although the possibility that these would have been detected with a larger sample size cannot be excluded. Nevertheless, two of these parasites scored probabilities of occurrence above 0.69, which is the average estimated for the four mainland blackcap parasites that were found on the islands (Fig. 3).

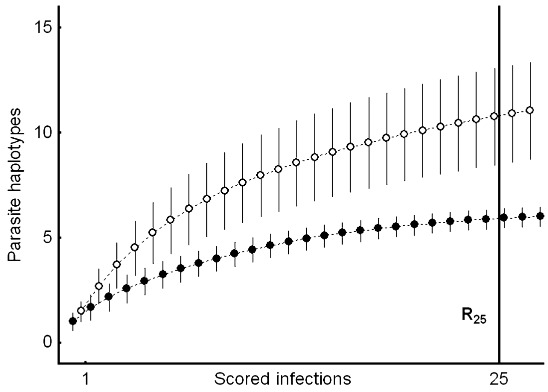

Figure 2 Cumulative parasite haplotype richness in insular (black dots) and mainland (open circles) blackcap populations. The curves represent mean richness (± SD) with increasing number of infections scored. The vertical line represents the sampling effort at which parasite richness was compared between islands and mainland.

Parasite richness was not correlated with the size of the blackcap's range on each island (Pearson's $r = 0.31$, $P = 0.60$; Spearman rank $r = 0.41$, $P = 0.49$; $n = 5$). However, we found a negative correlation between parasite richness and island distance to the continent (Fig. 4). When all data were considered, this effect was significant only when using nonparametric statistics (Pearson's $r = -0.83$, $P = 0.084$; Spearman rank $r = -0.97$, $P = 0.005$; $n = 5$). This correlation was clearer when tested considering only the more extensive data from 2007 (Pearson's $r = -0.83$, $r = -0.88$, $P = 0.047$; Spearman rank $r = -1$, $P < 0.001$; $n = 5$).

DISCUSSION

The assemblage of haemosporidian parasites found in Macaronesian blackcaps was very different from the one observed in mainland populations of the same species. The insular parasite assemblage was clearly impoverished, barely exceeding 10% of the parasite diversity known for mainland blackcaps. In addition, the structuring of the insular parasite assemblage was unusual, with *Leucocytozoon* dominating in terms of prevalence and haplotype richness, whereas in mainland Europe *Haemoproteus* is the predominant haemosporidian genus in blackcaps (Križanauskiene *et al.*, 2010; Santiago-Alarcón *et al.*, 2011; this study). As predicted, the island parasite assemblage was composed of parasites that were not specific to blackcaps, despite the fact that most parasites infecting continental blackcaps are host specific. Moreover, various of the parasites we found in insular blackcaps have already been shown to infect other species on the Atlantic Islands, despite the paucity of research on Macaronesian haemosporidians.

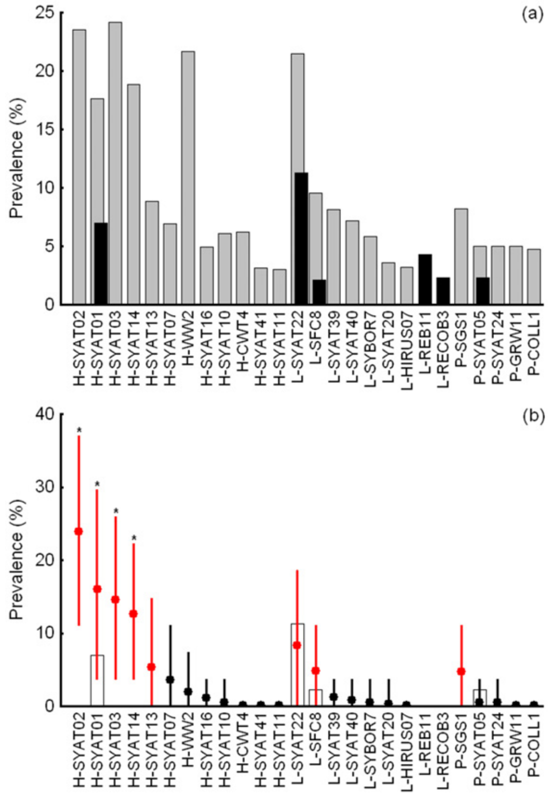

Figure 3 (a) Prevalence of haemosporidian parasites infecting blackcaps in the mainland populations (grey bars) and on islands (black bars), computed using populations/islands where each parasite occurred. (b) Expected island parasite prevalence of each mainland parasite (dots represent means, with 95% CI) computed from 10,000 Monte Carlo simulations. Based on individual probabilities of occurrence (P_{O}) obtained from these simulations, three parasites were predicted to occur on islands (marked with stars), and eight showed $P_{\mathrm{O}} > 0.69$ (in red), which is the average probability of occurrence computed for mainland parasites that were found on islands (their prevalence is shown with open bars). Parasite genera are distinguished by the initial of haplotype names (H: *Haemoproteus*, P: *Plasmodium*, L: *Leucocytozoon*).

Our observation, that parasite richness was uncorrelated with the size of blackcaps' range on each island, was to be expected given that the parasites identified were not blackcap specific, and such generalist parasites can rely on other host species to thrive. However, we found a negative correlation between distance from the continent and parasite richness. This correlation may result from various, not mutually exclusive processes: (1) sequential founder population bottlenecks in the parasites associated with the blackcaps (or other European host species) having first colonized the easternmost islands then spread westwards across islands (or with multiple colonization events affecting the easternmost islands more frequently; Dietzen *et al.*, 2008), (2) recurrent parasite colonization favoured by seasonal migration of blackcaps from Europe (which concentrate on the easternmost islands; Shirihai *et al.*, 2001), or (3) generalist parasites being transported from Africa and spreading westwards across the archipelago according to a stepping-stone pattern of island colonization. Whatever the mechanisms, our observation supports the basic tenet of island biogeography that the balance between colonization and extinction favours greater richness on

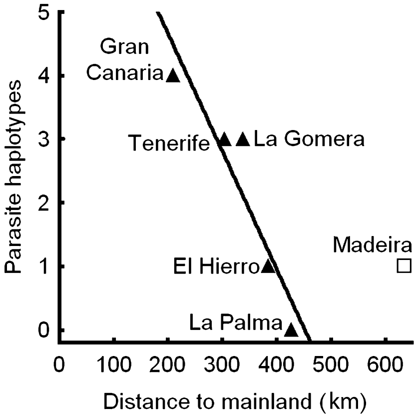

Figure 4 Correlation between parasite haplotype richness and island distance to the continent. The regression line was obtained excluding Madeira (open square), which is shown in the graph for comparative purposes only. Statistical analyses were performed with log-transformed values.

islands that are located near the putative source of colonizers (MacArthur & Wilson, 1967), a process that could also act indirectly on parasite diversity through its effects on hosts or vectors.

Together with the observed loss of parasite richness, overall parasite prevalence also drops from 65% on the mainland to around 10% on those islands that had parasites. This difference cannot be explained solely by the absence on islands of the many blackcap parasites that raise overall prevalence on the mainland, because all parasites that were shared between island and mainland blackcaps showed lower prevalence on islands. Apart from being less prevalent, parasites demonstrated apparent temporal instability in blackcap island populations, an observation that is at odds with previous studies showing temporal consistency in the structure of haemosporidian assemblages (Pérez-Tris & Bensch, 2005; Durrant *et al.*, 2008; Spurgin *et al.*, 2012; but see Bensch & Åkesson, 2003). Low and unstable parasite prevalence might indicate that parasites on the blackcaps on the islands are prone to extinction. This idea is supported by the composition of the parasite assemblage (comprising non-specific parasites capable of exploiting multiple hosts), which may benefit from the exploitation of alternative host species in years when particular hosts (blackcaps in our case) may for some reason be less available. Decreasing richness of parasites with increasing island distance to the continent (a putatively uninterrupted source of parasite colonization) may be evidence of frequent parasite extinction on islands, with parasite communities being re-seeded from the continent. Why parasite prevalence changes so much between years on islands and whether parasites persist in other host species during periods of low prevalence in blackcaps remain questions for future research.

An obvious candidate to explain the low richness and altered prevalence patterns observed on islands could be a reduced availability of appropriate vectors. This may be particularly relevant to the many blackcap-specific *Haemoproteus* haplotypes known from continental populations. *Haemoproteus* parasites are often vector specific (Martínez-de la Puente *et al.*, 2011), so the apparent inability of blackcap-specific parasites to thrive on islands could be associated with their specific vectors (*Culicoides* biting midges, Diptera: Ceratopogonidae) having failed to colonize Macaronesian archipelagos. If the midge species that have colonized the islands do not target blackcaps, or if they are not competent to transmit blackcap-specific *Haemoproteus* parasites, this would explain the absence of these parasites from the islands. Nevertheless, until the attributes of Macaronesian vector–bird–parasite interactions are thoroughly studied, we can only speculate on the possible role of vectors in driving the distribution of haemosporidians in insular blackcaps.

The only *Haemoproteus* parasite we found on insular blackcaps, *H. parabelopolskyi* H-SYAT01, is very common in mainland populations (Pérez-Tris & Bensch, 2005; Santiago-Alarcón *et al.*, 2011; this study), which could have favoured its transport to the islands. However, other parasites lineages with higher prevalence than H-SYAT01 in mainland blackcaps were not found on islands (most notably H-SYAT02; Pérez-Tris *et al.*, 2007). H-SYAT01 is known to also infect a related species, the African hill-babbler *Sylvia abyssinica*, an Afrotropical resident (Pérez-Tris *et al.*, 2007). Therefore, it is possible that H-SYAT01 might have benefited from its ability to thrive in hosts other than the blackcap, or from being transmitted by vectors of African origin. It is also worth noting that H-SYAT01 was only found in Gran Canaria, the insular blackcap population closest to the continent. This provides additional support for the idea that migrating blackcaps may permanently enhance parasite diversity on easternmost islands.

We found low diversity and prevalence of *Plasmodium* in the insular blackcap populations, with just two infections of *P. vaughani* P-SYAT05 on two different islands. This result was unexpected because, probably as a consequence of broad host and vector range, *Plasmodium* is the most abundant haemosporidian parasite genus on oceanic islands worldwide (Wikelski *et al.*, 2004; Beadell *et al.*, 2006; Howe *et al.*, 2012; Spurgin *et al.*, 2012). However, mainland blackcap populations do not show great prevalence or diversity of *Plasmodium* parasites. Therefore, given that parasite colonization success depends on the relative parasite frequency on the mainland (Fig. 3), the limited occurrence of this genus on the islands was to be expected. On the other hand, among *Plasmodium* haplotypes previously known for blackcaps P-SYAT05 was perhaps likely to be found on Macaronesia, because it is a host generalist that usually scores high prevalence in island avifaunas (Hellgren *et al.*, 2011; Ewen *et al.*, 2012).

The parasites of the genus *Leucocytozoon* found in insular blackcap populations offer interesting examples of parasite biogeography. Based on the abundant studies of European haemosporidians conducted so far (Bensch *et al.*, 2009), we can safely assume that L-SYAT22 behaves as a blackcap specialist in Europe. However, this is not the case in Macaronesia, where it also infects Berthelot's pipits (Spurgin *et al.*, 2012). The observation of L-SYAT22 in Berthelot's pipits may be evidence of ecological fitting (Agosta & Klemens, 2008). In this particular case, blackcaps could be acting as 'parasite reservoirs' from which L-SYAT22 spills over into other host species (a similar example is provided by blackbirds *Turdus merula* and their parasites on São Miguel Island, Azores; Hellgren *et al.*, 2011). Conversely, both L-REB11 and L-RECOB3 are *Leucocytozoon* haplotypes of putative tropical African adscription, which have been detected in several sedentary species in Nigeria. Outside mainland Africa, the haplotype L-REB11 has been found in Berthelot's pipits, while L-RECOB3 has been found in continental Europe (Ukraine) infecting garden warblers *Sylvia borin*, although only in adult individuals that had already visited tropical Africa (Hellgren *et al.*, 2007). After colonizing Macaronesia, these parasites had the opportunity to access host species of Palaearctic origin, e.g. blackcaps, as evidenced on our present study. Based on the previous records of these two parasites, their ability to infect a greater range of hosts may be constrained by strict environmental requirements or vectors that may not occur outside a narrow geographic range. Parasites like these are potential emergent disease threats if constraints on their range expansion are removed, for instance by vector species introductions or global warming (Patz *et al.*, 2000). In addition, in our study, these parasites represent clear cases of local parasite acquisition by a host species that lost other parasites after range expansion (Marzal *et al.*, 2011).

The broad picture unveiled in our study is that Macaronesian blackcaps have escaped the heavy parasite burden faced by their mainland counterparts. This may be partially interpreted as the likely outcome of 'missing the boat' (if parasites fail to arrive with its host to the new area) or 'drowning on arrival' (if they do arrive but fail to become established) processes (MacLeod *et al.*, 2010) during island colonization. However, blackcaps have not only lost their usual continental parasites after island colonization, they have also gained new parasites from those that were present in insular populations of other species. We are aware that these patterns cannot be completely understood without extensive knowledge of community-level bird–parasite interactions on the Macaronesian islands. However, our study adds to recent literature showing the impoverishment of insular parasite assemblages compared with mainland populations of the same host species (Hellgren *et al.*, 2011). In the blackcap, this pattern occurs despite the potential influx of diverse parasites through seasonal host migration. Therefore, our results support the idea that the typical characteristics of insular parasite assemblages (low lineage richness, frequent host-switching or reduced specialization of host–parasite relationships; Fallon *et al.*, 2005) may evolve before insular host populations become completely isolated from their mainland counterparts. Regardless of its causes, reduced parasite burden in insular populations of blackcaps and other species could have had evolutionary consequences (such as changes in bird immune resistance; Beadell *et al.*, 2007), which raise a cautionary word on the possible hazardous effects that the introduction of novel pathogens might have on yet another unique island bird community.

ACKNOWLEDGEMENTS

Roberto Carbonell, José Luis Tellería, Iván de la Hera, Juan Carlos Illera and Jean Secondi provided invaluable help during fieldwork. The Ministry of Environment of Portugal and the Spanish Regional Governments (Cabildos Insulares) kindly authorized fieldwork. The comments of David Currie and three anonymous reviewers greatly improved an early version of the manuscript. This study was funded by the Spanish Ministry of Science and Technology (grants CGL2007-62937/BOS and CGL2010-15734/BOS) and the Ministry of Education (FPU studentship to APR and postdoctoral fellowship to AR, EX-2006-0733). This is a contribution from the Moncloa Campus of International Excellence of the Complutense and the Polytechnic Universities of Madrid.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Table S1 Parasite richness and prevalence in Iberian blackcap populations.

BIOSKETCH

Antón Pérez-Rodríguez is a PhD student (supervised by Javier Pérez-Tris) interested in the biogeography of host–parasite relationships, with a special emphasis on the consequences of host migratory behaviour. He uses the blackcap and its associated haemosporidian parasites as a study model. APR, AR and JPT are members of the 'Vertebrate Biology and Conservation' group at Complutense University of Madrid, Spain (http://www.ucm.es/info/zoo/bcv_eng/index.html). DSR (University of East Anglia) is a collaborator of this group who also works the evolutionary ecology of avian systems across Macaronesia.

Author contributions: A.P.R. and J.P.T. conceived the study. J.P.T., A.R. and D.S.R. designed the sampling and conducted fieldwork. A.P.R. and J.P.T. performed laboratory analyses, analysed data and wrote the first draft of the manuscript. All authors improved the manuscript with their comments.

Editor: Shai Meiri