Journal of Biogeography (*J. Biogeogr.*) (2010) **37**, 120–132

ORIGINAL ARTICLE

Biogeographical patterns of blood parasite lineage diversity in avian hosts from southern Melanesian islands

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ABSTRACT

Aim (1) To describe the species–area relationships among communities of *Plasmodium* and *Haemoproteus* parasites in different island populations of the same host genus (Aves: *Zosterops*). (2) To compare distance–decay relationships (turnover) between parasite communities and those with potential avian and dipteran hosts, which differ with respect to their movement and potential to disperse parasite species over large distances.

Location Two archipelagos in the south-west Pacific, Vanuatu and New Caledonia (*c.* 250 km west of Vanuatu) and its Loyalty Islands, with samples collected from a total of 16 islands of varying sizes (328–16,648 km^2).

Methods We characterized parasite diversity and distribution via polymerase chain reaction (PCR) from avian (*Zosterops*) blood samples. Bayesian methods were used to reconstruct the parasite phylogeny. In accordance with recent molecular evidence, we treat distinct mitochondrial DNA lineages as equivalent to species in this study. Path analysis and parasite lineage accumulation curves were used to assess the confounding effect of inadequate sampling on the estimation of parasite richness. Species–area and species–distance relationships were assessed using linear regression: distance–decay relationships were assessed using Mantel tests.

Results Birds and mosquito species and *Plasmodium* lineages exhibited significant species–area relationships. However, *Plasmodium* lineages showed the weakest ‘species–area’ relationship; no relationship was found for *Haemoproteus* lineages. Avian species richness influenced parasite lineage richness more than mosquito species richness did. Within individual avian host species, the species–area relationship of parasites showed differing patterns. Path analysis indicated that sampling effort was unlikely to have a confounding effect on parasite richness. Distance from mainland (isolation effect) showed no effect on parasite richness. Community similarity decayed significantly with distance for avifauna, mosquito fauna and *Plasmodium* lineages but not for *Haemoproteus* lineages.

Main conclusions *Plasmodium* lineages and mosquito species fit the power-law model with steeper slopes than found for the avian hosts. The lack of species–distance relationship in parasites suggests that other factors, such as the competence of specific vectors and habitat features, may be more important than distance. The decay in similarity with distance suggests that the sampled *Plasmodium* lineages and their potential hosts were not randomly distributed, but rather exhibited spatially predictable patterns. We discuss these results in the context of the effects that parasite generality may have on distribution patterns.

Keywords

Distance–decay relationship, *Haemoproteus*, island biogeography, New Caledonia, *Plasmodium*, species–area relationship, Vanuatu, *Zosterops*.

www.blackwellpublishing.com/jbi
doi:10.1111/j.1365-2699.2009.02189.x

Journal of Biogeography

INTRODUCTION

Biogeographical patterns of parasitic organisms offer insights into the ecological processes that influence host–parasite associations, extinctions and colonizations (Apanius *et al.*, 2000). In a classic paper by Kuris *et al.* (1980), the general application of island biogeography theory to predict species richness of parasites or pests on hosts (as islands) was questioned on account of 'the unacceptable oversimplification' (p. 582) that such analysis entails. Island biogeography theory (MacArthur & Wilson, 1967) predicts that species richness will increase with the area of the island and this is commonly expressed as a power law

$$S = cA^z \qquad (1)$$

where S is the number of species, c is an empirically derived taxon- and location-specific constant, A is the area of the system sampled and z is the slope of the line. The relationship between species richness and island size has been estimated for many taxonomic groups including directly transmitted parasites (e.g. helminths, Dobson *et al.*, 1992; and mites, Lindström *et al.*, 2004), plants (Janzen, 1973), vertebrates, insects and bacteria (Wilson, 1961; Poulin, 1997; Green *et al.*, 2004; Horner-Devine *et al.*, 2004) but has never, to our knowledge, been estimated for vector-mediated parasites. Because of the dependence of vector-mediated parasites on multiple hosts for successful completion of their life cycle, there are several interesting questions regarding the influence of island isolation and size (see below). Further, whilst the decay in similarity with increasing distance between locations (i.e. beta diversity or turnover; Whittaker, 1975) has been widely explored in plant and animal communities (e.g. Nekola & White, 1999; Condit *et al.*, 2002), the decay in similarity of parasite communities has been explored for only a limited range of taxa (Poulin, 2003; Krasnov *et al.*, 2005; Vinarski *et al.*, 2007; Pérez-del-Olmo *et al.*, 2008; Seifertova *et al.*, 2008). Recent studies on host–parasite systems have indicated a tendency for species composition and/or richness to be spatially autocorrelated (Poulin & Morand, 1999; Poulin, 2003; Krasnov *et al.*, 2005). The ubiquity of the distance decay of similarity suggests the existence of similar mechanisms producing this pattern in different taxa. However, the generality of the pattern remains to be tested in blood parasite communities in which the environmental similarity for parasites involves not only the physical environment but also the ecological environment resulting from the species composition of the host and vector community.

Vector-transmitted blood parasites in the genera *Plasmodium* and *Haemoproteus* are widespread and cosmopolitan (except for Antarctica) and have been detected in many avian species (Valkiūnas, 2005). Recent application of molecular genetic techniques to the detection of blood parasites has revealed a wealth of genetic diversity among parasites (Bensch *et al.*, 2000; Perkins & Schall, 2002; Beadell *et al.*, 2004), much of which was not evident from morphology alone (Beadell *et al.*, 2006). In addition, the majority of distinct lineages identified from mitochondrial DNA sequences have been shown to represent reproductively isolated entities – effectively biological species (Bensch *et al.*, 2004; Hellgren *et al.*, 2007). While we refer to them as lineages, we treat them as if they are biological species for the remainder of this paper (that is, they are our unit of analysis). *Haemoproteus* lineages are considered more host-specific than *Plasmodium* lineages, probably owing to their high vector fidelity on ceratopogonid and hippoboscid flies. Avian *Plasmodium* lineages primarily exploit culicine mosquito species (Valkiūnas, 2005) and have been shown to exhibit extreme generalist host–parasitism strategies (Beadell *et al.*, 2009). Given the complex life cycles of these two haematozoan genera, the distribution of lineages and patterns of diversity should be subject to a suite of influences, such as vector and host distributions as well as biogeographical parameters resulting from island area and isolation. The level of phylogeographical structure will depend on the factors that strongly influence parasite distribution; if avian host distribution is the primary factor, then a patchy distribution of parasite lineages among host populations across islands is expected. If other factors, such as vector diversity and habitat heterogeneity have a stronger influence, then phylogeographical structure may develop in parasite lineages.

The shape of species–area, species–isolation and distance–decay relationships may also depend on whether parasites are generalist or specialist with respect to their vector and host associations. Parasites with specialized vector associations tend to have a restricted host range (Killick-Kendrick, 1978) and therefore may be limited by the geographical distribution of a specific host (Lajeunesse & Forbes, 2002). In contrast, the use of a broad spectrum of blood-feeding vectors should facilitate host switching in generalist parasites (Githeko *et al.*, 1994). Additionally, parasites with broad host distributions may achieve higher abundance relative to specialists; therefore one might expect lower slope and weaker distance–decay rates of community composition for generalist than for specialist parasites. In addition, the geographical isolation of islands may not be a limiting factor in vector-mediated parasites; instead, availability of competent vectors or suitable environmental variables might play a more important role in the distribution of specialist parasites. The patterns observed may also be influenced by the choice of host species that are sampled and their respective colonization histories. For example, if islands are invaded multiple times from different source locations by avian hosts (e.g. *Zosterops* species on south-west Pacific islands; Mees, 1969; Phillimore *et al.*, 2008), a diverse array of parasites may be introduced. The colonizing hosts may also be susceptible to newly encountered parasites (Combes, 2001, pp. 112–146). Both of these factors may lead to higher parasite richness and stronger species–area relationships in colonized as compared with endemic hosts.

Using mitochondrial DNA sequence data to classify parasites to lineages, we investigated biogeographical patterns of *Plasmodium* and *Haemoproteus* lineages in small passerines (family: Zosteropidae) sampled across two Melanesian

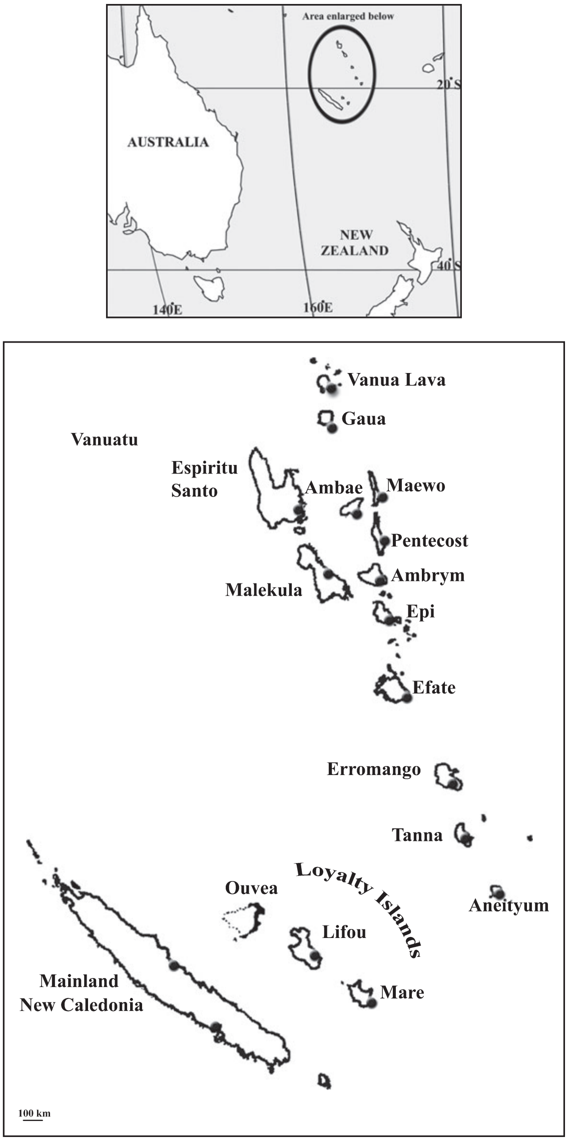


Figure 1 *Zosterops* sampling sites (dots) in Vanuatu and New Caledonia employed for this study. See Table 1 for sample sizes.

archipelagos: Vanuatu and New Caledonia (Fig. 1). These archipelagos are ideal locations for this study because the spatial distribution of dipteran and avian hosts is well characterized (Mayr, 1942; Belkin, 1962; Bregulla, 1992) and the potential role of mosquito species in parasite transmission has been explored (Ishtiaq *et al.*, 2008). Because members of the silvereye (*Zosterops lateralis*) species complex co-occur with different endemic *Zosterops* species across multiple archipelagos, this is an ideal system for comparing parasite assemblages within hosts and island groups. The aims of this study were: (1) to characterize the diversity and distribution of *Plasmodium* and *Haemoproteus* lineages across the host species and islands; (2) to examine the species–area relationships for both parasite genera and compare them with those of their potential invertebrate and vertebrate hosts; and (3) to test whether the similarity in parasite lineage composition decays with geographical distance and compare this relationship with the analogous measure in their vertebrate and invertebrate hosts.

MATERIALS AND METHODS

Sample collection and preparation

Avian system

The islands within the Vanuatu archipelago are inhabited by two *Zosterops* species: the endemic yellow-fronted white-eye (*Zosterops flavifrons*) (Gmelin, 1789) and the later colonizing silvereye (*Z. lateralis*) (Latham, 1802) (Mees, 1969). The latter also occurs on all islands of New Caledonia along with three endemic species of white-eye: the green-backed white-eye (*Zosterops xanthochrous* Gray, 1859) on mainland New Caledonia and Mare Island, and the small Lifou white-eye (*Zosterops minutus* Layard, 1878) and the large Lifou white-eye (*Zosterops inornatus* Layard, 1878), both restricted to Lifou. Two species of *Zosterops* were sampled from 13 of the largest islands in the Vanuatu archipelago (*c.* 1900 km east of Australia; Fig. 1). Aneityum and Maewo are the only islands sampled where *Z. flavifrons* is present but *Z. lateralis* is currently absent. A total of 208 samples of *Z. lateralis* and 340 samples of *Z. flavifrons* were collected by A.B.P. (February–May 2004) and S.M.C. (February–April 2006). In New Caledonia (*c.* 250 km west of Vanuatu), 155 *Zosterops* samples were collected from the mainland and the Loyalty Island group by R.A.B. (September–December 2006): *Z. lateralis* ($n = 60$), *Z. xanthochrous* ($n = 60$), *Z. minutus* ($n = 25$) and *Z. inornatus* ($n = 10$). Parasite prevalence has been shown to vary by gender and age in other avian systems (e.g. Ots & Horak, 1998) and to be high during the breeding season, perhaps due to reduced allocation to immune defences (Sheldon & Verhulst, 1996). We have not included gender as a variable in this study, as detailed observations of one island population of *Zosterops* have shown that both members of a pair contribute to territory defence, nest building, incubation and feeding chicks and fledglings – in other words that gender roles are similar (Kikkawa & Wilson, 1983). Bird samples consisted of juveniles and adults caught towards the end of the breeding season in Vanuatu, and prior to the breeding season in New Caledonia. Nestlings or fledglings were not sampled. Birds were caught using mist nets or traps and 20–40 μL of blood from the subbrachial wing vein was sampled and stored in 70% ethanol or on wet strength no. 13 Whatman filter paper.

Parasite screening and phylogenetic analysis

The presence of *Plasmodium* and *Haemoproteus* was assessed using parasite-specific primers designed to amplify the cytochrome *b* gene fragments (see Appendix S1 in Supporting Information for detailed laboratory methods and phylogenetic analysis).

Statistical analyses

Parasite prevalence and diversity

We used contingency table analyses to test for the heterogeneity in parasite prevalence across host species and islands using SPSS version 9.0. Generalized linear models (GLMs) were used with a binomial error and correction for over-dispersion in SAS version 8.2, to test whether the host or island predicted parasite prevalence. Heterogeneity in parasite lineage distributions was assessed by *G*-tests followed by partitioned analyses (Sokal & Rohlf, 1995) in SPSS version 9.0.

Parasite richness and species–area and species–distance relationships

Since parasite richness and island size might both correlate with sampling effort (Rosenzweig, 1995; Walther *et al.*, 1995), we employed path analysis (Sokal & Rohlf, 1995) to explore the direct and indirect effects of island size and the sample size of hosts on overall estimates of parasite richness as suggested by Guégan & Kennedy (1996) (see Appendix S2). We supplemented this analysis using EstimateS version 8.0 (Colwell, 2006), and the nonparametric Chao2 estimator (S^*_2 = mean ± SD) (Colwell & Coddington, 1994), to calculate the cumulative parasite lineage richness (by adding a new parasite lineage as each new lineage was detected in an infected host). This estimated our success in sampling the available lineages of haematozoan taxa from all island populations and hosts.

Using equation 1, we assessed the relationship between parasite species richness (using $n + 1$ for some species number values, as some islands had zero species; see Buskirk, 1985) and island size with a least-squares regression analysis on log-transformed variables. This analysis was conducted across all *Zosterops* species considered together, and then separately within each of the two widespread species, *Z. lateralis* and *Z. flavifrons*. Subsequently, we assessed the species–area relationship between species richness of avian hosts and mosquito species and island size. A path analysis was employed to assess if either avian or mosquito richness predicted parasite richness.

The species–distance relationship (degree of isolation) was assessed using least-squares multiple regression including island size and distance as predictors of species richness in SPSS version 9.0. The size of the islands was retrieved from http://islands.unep.ch/isldir.htm (accessed April 2009) and ranged from 334 to 16,648.4 km^2, and the distance from the nearest continental landmass (Australia) ranged between 1416.93 and 2127 km (retrieved via Google Earth version 4.2). Australia is considered as a major source for *Zosterops* colonizations across these archipelagos (Mees, 1969).

Distance–decay relationship

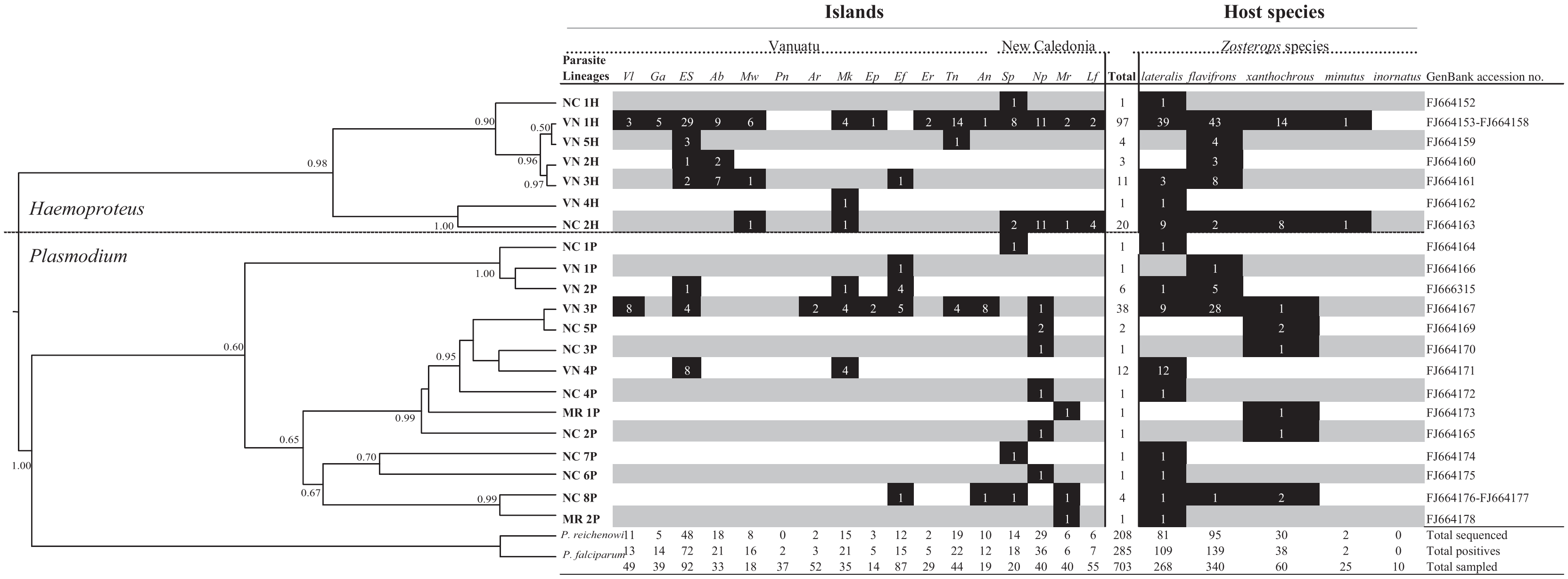
Data on the presence and absence of birds and mosquito species and parasite lineages were used to calculate the Sørensen index (Legendre & Legendre, 1998) to explore beta-diversity patterns. To calculate the significance of the correlation between log-transformed Sørensen similarity coefficients and geographical distance for each pair of samples, we applied Mantel tests (Sokal & Rohlf, 1995) with 9999 permutations, implemented in SAS version 8.2. The similarity coefficients were calculated on square root-transformed data to decrease the influence of highly dominant lineages. Some similarity values were equal to zero (i.e. there were no species in common), therefore we coded the similarity data by adding 0.01 before log-transforming each value (Legendre & Legendre, 1998). Information on the distribution of avian species (n = 151) was obtained from Bregulla (1992), the Avibase world bird database (http://avibase.bsc-eoc.org/; accessed April 2009) and Vanbirds database (Wantoc Environment Centre, http://www.positiveearth.org/vanbirds/; accessed April 2009). Mosquito species (n = 34) distributions for each island group were obtained from Belkin (1962).

RESULTS

Using polymerase chain reaction (PCR) we detected haematozoan infections in 285 out of 703 individuals (40.5%) from the five *Zosterops* species sampled. Of the positive samples, 29.1% (83) were *Plasmodium*, 66.6% (190) were *Haemoproteus* and 4.2% (12) were mixed infections (i.e. infected with both). A total of 208 readable mitochondrial DNA sequences provided genus-specific variation in parasite prevalence. Parasite prevalence showed no significant difference between the three sampling periods for *Zosterops* species: February–May 2004, February–April 2006 and October–December 2006 (χ^2 = 2.21, d.f. = 2, P = 0.33). We therefore combined the prevalence data for the three sampling periods. The *Plasmodium* and *Haemoproteus* lineages recovered as part of this study fall into two well-supported clades (Fig. 2). We identified 21 distinct parasite lineages, of which 14 were *Plasmodium* and seven were *Haemoproteus* lineages.

Prevalence and distribution of parasite lineages in vertebrate hosts and islands

There was considerable heterogeneity in parasite prevalence across the five host species, ranging from 0% to 13% for *Plasmodium* and 0% to 48% for *Haemoproteus*. None of the *Z. inornatus* samples (n = 10) showed evidence of infection. Across the two archipelagos, *Plasmodium* was not detected from four of the 16 islands examined, whilst *Haemoproteus* was found on all islands. Island-wide prevalence of parasites varied across the two archipelagos for both *Plasmodium* (χ^2_{15} = 81.31, P < 0.0001) and *Haemoproteus* (χ^2_{15} = 257.32, P < 0.0001) (Appendix S3). *Plasmodium* prevalence was higher in New Caledonia than Vanuatu, whereas *Haemoproteus* lineages were dominant in Vanuatu. Furthermore, the distribution of specific lineages within each parasite genus demonstrated significant heterogeneity in prevalence across the archipelagos (Fig. 2). Lineage VN 3P, for example, showed



	Islands																		**Host species**					
	Vanuatu													New Caledonia					*Zosterops* species					
Parasite Lineages	*Vl*	*Ga*	*ES*	*Ab*	*Mw*	*Pn*	*Ar*	*Mk*	*Ep*	*Ef*	*Er*	*Tn*	*An*	*Sp*	*Np*	*Mr*	*Lf*	**Total**	*lateralis*	*flavifrons*	*xanthochrous*	*minutus*	*inornatus*	GenBank accession no.
NC 1H														1				1	1					FJ664152
VN 1H	3	5	29	9	6			4	1		2	14	1	8	11	2	2	97	39	43	14	1		FJ664153-FJ664158
VN 5H			3									1						4		4				FJ664159
VN 2H			1	2														3		3				FJ664160
VN 3H			2	7	1					1								11	3	8				FJ664161
VN 4H								1										1	1					FJ664162
NC 2H					1			1						2	11	1	4	20	9	2	8	1		FJ664163
NC 1P														1				1	1					FJ664164
VN 1P										1								1		1				FJ664166
VN 2P			1					1		4								6	1	5				FJ666315
VN 3P	8		4				2	4	2	5		4	8		1			38	9	28	1			FJ664167
NC 5P															2			2			2			FJ664169
NC 3P															1			1			1			FJ664170
VN 4P			8					4										12	12					FJ664171
NC 4P															1			1	1					FJ664172
MR 1P																1		1			1			FJ664173
NC 2P															1			1			1			FJ664165
NC 7P														1				1	1					FJ664174
NC 6P															1			1	1					FJ664175
NC 8P										1			1	1		1		4	1	1	2			FJ664176-FJ664177
MR 2P																1		1	1					FJ664178
P. reichenowi	11	5	48	18	8	0	2	15	3	12	2	19	10	14	29	6	6	208	81	95	30	2	0	Total sequenced
P. falciparum	13	14	72	21	16	2	3	21	5	15	5	22	12	18	36	6	7	285	109	139	38	2	0	Total positives
	49	39	92	33	18	37	52	35	14	87	29	44	19	20	40	40	55	703	268	340	60	25	10	Total sampled

Figure 2 Phylogeny of *Plasmodium* and *Haemoproteus* lineages recovered from *Zosterops* samples from two Melanesian archipelagos and based on the cytochrome *b* gene (271–351 bp). Maximum clade credibility tree from Bayesian relaxed clock analysis. Support values indicate posterior clade probabilities. The tree was rooted using two mammalian parasites. Islands are arranged in geographical order from north to south: *Vl*, Vanua Lava; *Ga*, Gaua; *ES*, Espiritu Santo; *Ab*, Ambae; *Mw*, Maewo; *Pn*, Pentecost; *Ar*, Ambrym; *Mk*, Malekula; *Ep*, Epi; *Ef*, Efate; *Er*, Erromango; *Tn*, Tanna; *An*, Aneityum; *Sp*, South Province; *Np*, North Province (both provinces constitute mainland New Caledonia); *Mr*, Mare; *Lf*, Lifou.

high prevalence in islands at the northern and southern ends of the Vanuatu archipelago, but lower prevalence and a rather patchy distribution elsewhere in Vanuatu, and was recorded only once in New Caledonia (*G*-test of heterogeneity: $G = 52.4$, d.f. = 15, $P < 0.001$). Likewise, lineage VN 1H had a wide geographical distribution but was patchy in terms of prevalence across both archipelagos ($G = 39.5$, d.f. = 15, $P < 0.001$). Lineage NC 2H was prevalent in New Caledonia but was represented only twice from two central islands in Vanuatu ($G = 24.5$, d.f. = 15, $P < 0.05$). In the remaining cases, lineages were distributed rather sparsely, or were recorded only once in either archipelago (Fig. 2).

Across the populations of *Z. lateralis* and *Z. flavifrons* that coexist on islands within the Vanuatu archipelago (except Maewo and Aneityum), a GLM with binomial errors and correction for over-dispersion revealed that prevalence of *Plasmodium* differed significantly among islands ($\chi^2_{10} = 24.49$, $P < 0.0064$), but did not differ significantly between the two *Zosterops* species ($\chi^2_1 = 0.13$, $P = 0.72$). Similarly, for *Haemoproteus*, there was a highly significant effect of island ($\chi^2_{10} = 177.16$, $P < 0.0001$), and no effect of *Zosterops* species ($\chi^2_1 = 0.117$, $P = 0.73$). Across all *Zosterops* species, *Plasmodium* showed no significant differences in prevalence among sampling sites ($\chi^2_4 = 5.11$, $P = 0.27$), whereas the prevalence of *Haemoproteus* differed significantly ($\chi^2_4 = 22.46$, $P < 0.0001$).

Parasite richness, species–area and species–distance relationships

We found no evidence that uneven sampling efforts biased estimates of parasite richness across islands and host species: the path analysis showed non-significant path coefficients associated with sampling (see Appendix S2). Table 1 gives details of parasite richness and the Chao2 (S^*_2 = mean ± SD) estimates for each host species, each island and each archipelago. *Zosterops lateralis* showed the highest parasite richness (66%) and the largest proportion (33%) of exclusive parasites found among the five host species. Despite having the highest parasite richness, Chao2 estimated a higher number of parasite lineages than actually detected ($S_{\text{obs}} = 14$, $S^*_2 = 23.58 \pm 14.99$). *Zosterops flavifrons* harboured 43% of the total parasites and 14% of the exclusive parasite lineages. The Chao2 estimate of species richness was similar to the number of lineages detected ($S_{\text{obs}} = 9$, $S^*_2 = 8.03 \pm 1.90$). Mainland New Caledonia was exceptionally rich in parasites (76%), with an accumulation curve that estimated more parasite lineages than detected ($S_{\text{obs}} = 12$, $S^*_2 = 21.76 \pm 12.10$; Table 1, Appendix S4). However, across the entire New Caledonian archipelago, the accumulation curve reached an asymptote ($S_{\text{obs}} = 14$, $S^*_2 = 8.99 \pm 5.19$). Vanuatu populations harboured 62% of parasites and the accumulation curve reached an asymptote ($S_{\text{obs}} = 11$, $S^*_2 = 11.78 \pm 2.58$).

Species–area relationships varied among parasites, birds and mosquito hosts (Table 2). Among all *Zosterops* hosts, significant species–area relationships were found for overall parasite richness and within *Plasmodium* but not for *Haemoproteus*. These significant relationships disappeared when the largest island (mainland New Caledonia) was excluded. Species–area relationships of parasites found in *Z. lateralis* were significant both overall and when *Plasmodium* and *Haemoproteus* lineages were considered separately (Fig. 3, Table 2a). There were no significant species–area relationships evident within the parasites of the endemic *Z. flavifrons* from Vanuatu (Table 2a). The species richness of mosquito species exhibited a relatively steep relationship with island area (Table 2b), and a strong relationship was also evident in avian hosts (Table 2c; see also Appendix S5).

Table 1 Sampling effort and observed and expected lineage richness for avian haematozoa across two Melanesian archipelagos. The expected values (S^*_2) are based on lineage accumulation curves in each island population and host species. S^*_2(mean ± SD) represents Chao2 estimator with mean and standard deviation.

Groups	Sample size	*n* lineages (S_{obs})	S^*_2 = mean ± SD	Exclusive lineages
Host species				
Zosterops lateralis	268	14	23.58 ± 14.99	7
Zosterops flavifrons	340	9	8.03 ± 1.90	3
Zosterops xanthochrous	60	8	10.22 ± 6.91	5
Zosterops minutus	25	2	1.52 ± 0.77	0
Islands				
Vanuatu				
Vanua Lava	49	2	1.66 ± 0.22	0
Gaua	39	1	0.81 ± 0.13	0
Espiritu Santo	92	7	5.74 ± 1.49	0
Ambae	33	3	2.60 ± 0.41	0
Maewo	18	3	2.48 ± 1.15	0
Pentecost	37	0	0	0
Ambrym	52	1	0.70 ± 0.11	0
Malekula	35	6	5.55 ± 2.29	1
Epi	14	2	1.43 ± 0.39	0
Efate	87	5	4.81 ± 2.72	0
Erromango	29	1	0.64 ± 0.10	0
Tanna	44	3	2.34 ± 0.43	0
Aneityum	19	3	2.49 ± 1.09	0
New Caledonia				
South Province*	20	6		3
North Province*	40	8		4
Mainland NC	60	12	21.76 ± 12.10	7
Mare	40	5	4.72 ± 2.71	2
Lifou	55	2	1.70 ± 0.47	0
Archipelago				
Vanuatu	548	11	11.78 ± 2.58	1
New Caledonia	155	14	8.99 ± 5.19	9

*Mainland NC = New Caledonia.

A path analysis showed that parasite lineage richness was significantly correlated with all variables (avian species richness, mosquito species richness and island area), ($r^2 = 0.73$, F (3, 12) = 4.78, $P < 0.020$), but the partial effects of each variable fell short of statistical significance

Table 2 Species–area relationships for (a) parasites, (b) mosquitoes and (c) birds in Melanesian archipelagos.

	All parasites	*Plasmodium*	*Haemoproteus*
(a) Parasites			
All *Zosterops*	**$z = 0.35$, $r^2 = 0.40$, $P < 0.009$**	**$z = 0.40$, $r^2 = 0.40$, $P < 0.008$**	$z = 0.096$, $r^2 = 0.14$, $P = 0.14$
All *Zosterops**	$z = 0.29$, $r^2 = 0.18$, $P = 0.16$	$z = 0.29$, $r^2 = 0.12$, $P = 0.11$	$z = 0.11$, $r^2 = 0.028$, $P = 0.25$
Zosterops lateralis only	**$z = 0.46$, $r^2 = 0.60$, $P < 0.001$**	**$z = 0.52$, $r^2 = 0.57$, $P < 0.001$**	**$z = 0.24$, $r^2 = 0.28$, $P < 0.04$**
Zosterops flavifrons only	$z = 0.05$, $r^2 = 0.025$, $P = 0.73$	$z = 0.03$, $r^2 = 0.003$, $P = 0.84$	$z = 0.007$, $r^2 = 0.003$, $P = 0.96$
(b) Mosquito species	**$z = 0.49$, $r^2 = 0.40$, $P < 0.008$**		
(c) Bird species	**$z = 0.23$, $r^2 = 0.75$, $P < 0.0001$**		

Significant relationships are shown in bold.
z, Slope; r^2, regression correlation coefficient; P, P-value.
*Excluding mainland New Caledonia.

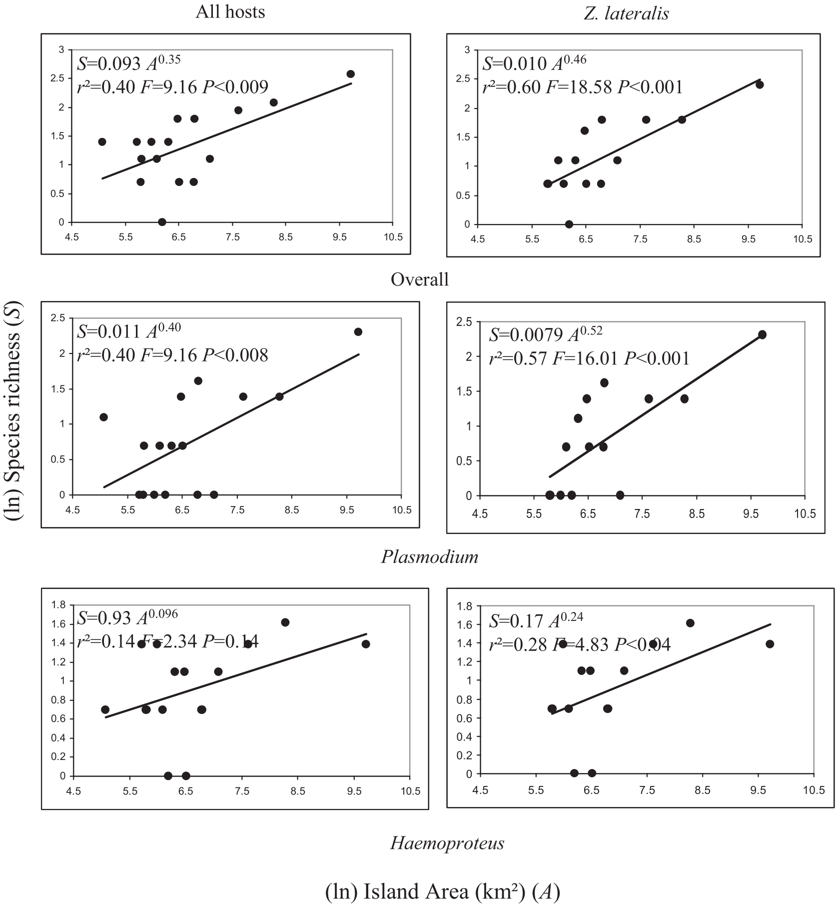


Figure 3 Species–area relationships of *Plasmodium* and *Haemoproteus* lineages in *Zosterops* (all species) on the left side and in *Zosterops lateralis* on the right side.

($P > 0.05$). This may be due to redundancy of information caused by the high correlation among the variables; individually, each variable significantly influenced parasite richness (Fig. 4). When the island area variable was excluded from the model, the beta weight for avian richness was markedly higher (0.65; $P < 0.033$) than that for mosquito richness (0.13; $P = 0.63$).

Multiple regression analysis on the relationship of parasite richness with respect to island area and distance (isolation) showed that overall parasite richness and *Plasmodium* lineage richness was positively correlated with island area ($r^2 = 0.60$, F (2, 11) = 9.06, $P < 0.005$), with significantly high beta weight (0.66, $P < 0.05$) but not with distance ($P = 0.52$), suggesting that factors other than isolation may be important. However, *Haemoproteus* lineage richness showed no correlation ($r^2 = 0.30$, F (2, 11) = 2.23, $P = 0.15$) with either island area ($P = 0.19$) or distance from mainland ($P = 0.87$). It made no qualitative difference if mainland New Caledonia rather than Australia was considered as the closest potential source population (results not shown).

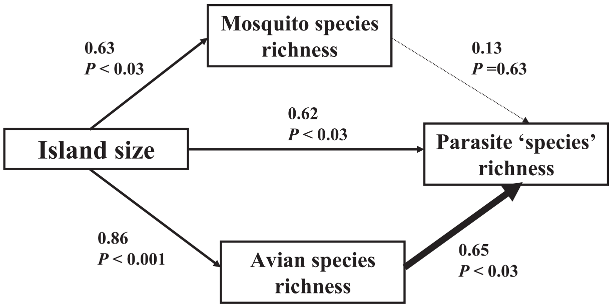


Figure 4 Path analysis to predict the effect of avian species richness and mosquito species richness on the overall parasite richness. The numbers beside the arrows show the direct and indirect effects of path coefficients. The thickness of the arrows shows the significance of the relationship.

Table 3 Summary statistics results of distance–decay relationships in bird species, mosquito species and *Plasmodium* and *Haemoproteus* lineages across two Melanesian archipelagos.

Taxa	Islands	*n*	Intercept	Slope	r^2	*P*
Birds	16	120	1.055	−0.287	0.5	0.0001
Mosquitoes	16	120	0.414	−0.417	0.04	0.039
Plasmodium	10	45	3.247	−0.783	0.15	0.011
Haemoproteus	14	91	−1.471	0.085	0.002	0.4

n, the corresponding number of similarity pairs (shown in dots in Fig. 5); r^2 represents the regression statistic based on 9999 permutations.

Distance–decay relationship

Among the four taxonomic groups across islands, community similarity declined significantly with geographical distance for bird species, mosquito species and *Plasmodium* lineages (Table 3, Fig. 5). The slope for birds was less than for mosquitoes, which in turn was only half that for *Plasmodium* lineages. In contrast, lineage composition of *Haemoproteus* showed no significant relationship with increasing geographical distance between islands.

DISCUSSION

A biogeographical survey of the parasite lineage richness and community composition in southern Melanesia revealed first, that parasite prevalence and diversity showed heterogeneous distribution across hosts and islands. Second, birds and mosquito species and *Plasmodium* lineage richness increased with island area as predicted by the power law; however, *Plasmodium* lineage richness exhibited a steeper slope than did the richness of the vertebrate and invertebrate hosts. In contrast, *Haemoproteus* showed no relationship between lineage richness and area and there was no evidence that distance from the mainland (isolation) had an effect on the parasite communities. Lastly, the distance–decay relationship showed a predictable pattern in community composition of avian, mosquito species and *Plasmodium* lineages, while community composition of *Haemoproteus* lineages showed

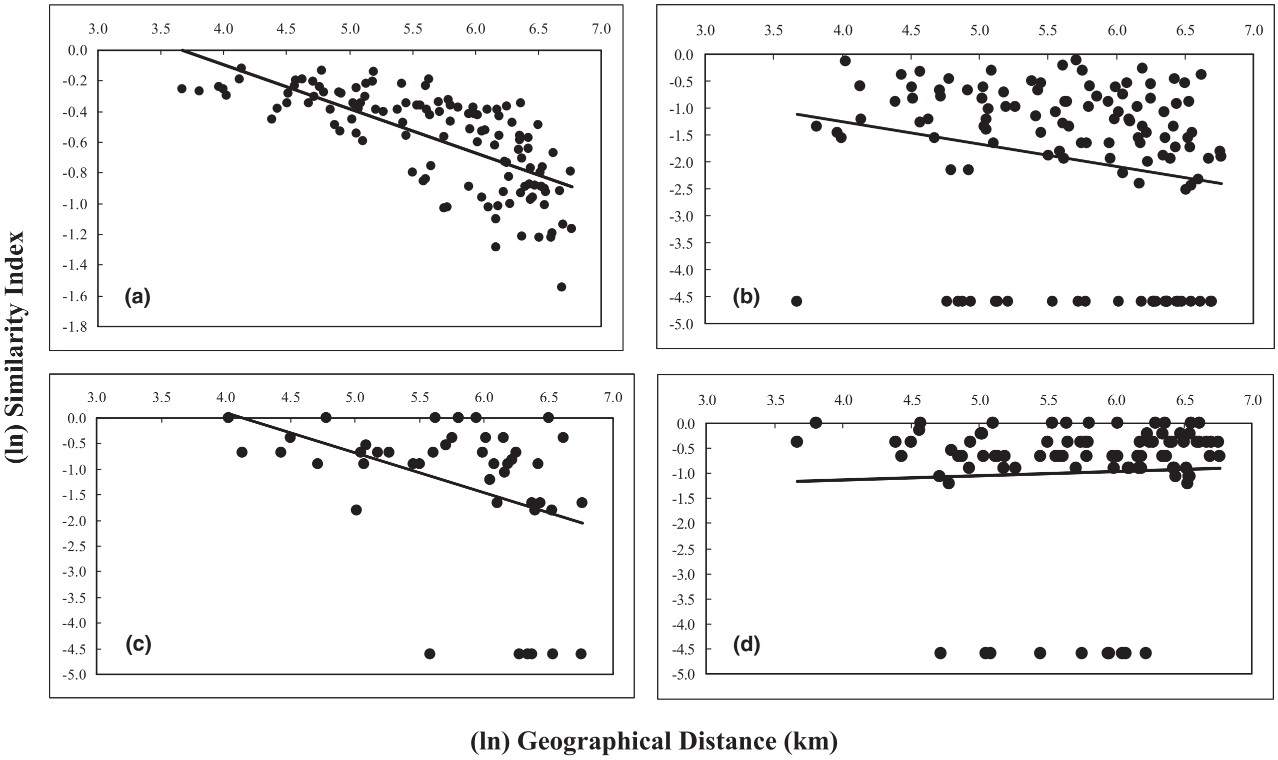


Figure 5 Relationship between pairwise geographical distance between Melanesian islands and similarity in community composition: (a) birds ($P < 0.001$); (b) mosquitoes ($P < 0.04$); (c) *Plasmodium* ($P < 0.011$); (d) *Haemoproteus* ($P = 0.40$), based on 9999 permutations. Points at −4.6 are pairwise comparisons where no species were shared in common.

no significant relationship with distance. Here, we discuss the factors that contribute to these different patterns in two vector-mediated parasites and compare their turnover with potential host species along a spatial gradient.

Prevalence, diversity and distribution of parasite lineages

Islands clearly influence the prevalence and distribution of individual parasite lineages whereas seasonal effects have been shown to influence the presence of parasites in hosts (e.g. Cosgrove *et al.*, 2008). In tropical climates, avian malaria occurs throughout the year (Valkiūnas, 2005), whereas studies in temperate regions report consistent seasonal variation with a peak in prevalence during spring or the breeding season when vector populations and the proportion of immunologically naïve juveniles in the host population are high and higher levels of sexual steroid hormones depress the immune system, allowing parasites to survive (Wedekind & Følstad, 1994; Saino *et al.*, 1995). This is followed by a decline during winter (Schrader *et al.*, 2003; Cosgrove *et al.*, 2008) as vector activity decreases and malaria parasites disappear from the blood, but may remain in body tissues, followed by a spring relapse of infection prior to the breeding season. The tropical climate of Melanesian islands, combined with the sampling of birds towards the end of the breeding season, may have increased the likelihood of detecting these parasites at a relatively high prevalence in the sampled avian host populations.

Three of the 21 parasite lineages detected here could be considered to be generalist parasites with wide host and geographical distributions. Other lineages were encountered once and/or restricted to particular islands or endemic hosts, and could thus be considered to be specialist lineages. The heterogeneous nature of prevalence and distribution, despite the often continuous distribution of host species, suggests that there are constraints on the distribution of particular parasite lineages within the system. Parasite distributions are likely to be strongly influenced by vector–parasite interactions and the distribution of competent vectors (Sol *et al.*, 2000). Ishtiaq *et al.* (2008) have demonstrated close associations between potentially specialist parasite lineages and endemic mosquito species, for example two *Plasmodium* lineages (LIN 2p equivalent to VN 4P and LIN 3p equivalent to MR 1P in this study) in Vanuatu and New Caledonia, respectively. This implies that the movement of parasite lineages between islands may be restricted by the lack of overlap in distribution range of competent vector species. Archipelagos are probably quite vulnerable to stochastic events, such as chance colonization or extinction, which will play an important role in the evolution of local parasite assemblages.

There was a tendency for *Haemoproteus* lineages to show higher host sharing among *Zosterops* species than *Plasmodium*, with an average of two *Zosterops* species infected per *Haemoproteus* lineage, compared with 1.35 *Zosterops* species per *Plasmodium* lineage. This is consistent with other studies where *Haemoproteus* shared more lineages between closely related host species than *Plasmodium* (Bensch *et al.*, 2000; Ricklefs & Fallon, 2002; Beadell *et al.*, 2004). While both parasite genera have a heterogeneous distribution across Vanuatu and New Caledonia, our data suggest that geographical structuring is stronger for *Plasmodium* than *Haemoproteus* (Figs 2 & 5). This is consistent with patterns of haematozoan diversity in the Caribbean islands, which showed strong geographical structuring among *Plasmodium* lineages contrasting with a more even distribution of *Haemoproteus* lineages throughout the archipelago (Fallon *et al.*, 2003).

Species–area and species–distance relationships

Birds and mosquito species and *Plasmodium* lineage richness increased with island size whereas *Haemoproteus* lineage richness exhibited no such relationship. Within *Z. lateralis* hosts, *Plasmodium* and overall parasite richness followed the species–area power law. Islands of similar sizes yielded similar estimates of species richness regardless of the distances between them. Interestingly, in *Z. lateralis*, the z-values for *Plasmodium* (0.52) fall above, and that for overall parasite richness (0.46) just within, the upper limit of the 'normal' range of values observed in most taxa on islands ($0.25 < z < 0.48$; Rosenzweig, 1995). Hence, these parasites have a steeper species–area curve relative to their avian hosts as a whole (0.23), and are rather closer to that of their invertebrate hosts (0.49). The relatively high z-values for *Plasmodium* in *Z. lateralis* and invertebrate hosts are generally consistent with the z-values that would be expected to occur in distant archipelagos with low immigration rates (MacArthur & Wilson, 1967). Low immigration rates for parasites could be the result of habitat heterogeneity and other environmental variables, which might act as barriers to colonization by decreasing the movements of invertebrate hosts (Lum *et al.*, 2007). Because of this increasing isolation effect, species–area curves decline in richness with reduced c-value and become steeper with increased z-value (Lomolino, 2001). The steeper species–area relationship that emerged for *Plasmodium* does not fit very well with the species–area predictions for generalist parasites, in which frequent host-switching may facilitate wider geographical distribution relative to specialists.

The lack of a species–area relationship in *Haemoproteus* may be due to limited host sampling combined with high specificity to host families resulting in the higher *Haemoproteus* prevalence and wider distribution across these islands. The predicted effect of avian species richness on parasite richness supports this suggestion; sampling from a broad range of avian hosts may therefore reveal a greater diversity of lineages. The overall species–area patterns for parasites in this study were driven by sampling from the more recent colonist (*Z. lateralis*). The larger the biogeographical area of the host species, the more diverse that host's parasite fauna; high z-values typify those that come from distinct biogeographical provinces (Rosenzweig, 1995; Triantis *et al.*, 2008). However, high parasite diversity in *Z. lateralis* was probably not simply due to its large geographical range, which may have increased the possibility of exposure to a wide array of parasite assemblage (Combes, 2001, pp. 112–146). There is a higher likelihood of colonization by local parasites for an invading species in a new region that contains phylogenetically and ecologically similar species than hosts that become established in areas lacking similar species (Torchin *et al.*, 2003). In addition, populations acquiring novel parasites will often lack effective immune defences against new parasites making them more susceptible to infection (Anderson *et al.*, 2004). Given phylogenetic estimates that *Z. lateralis* colonized Vanuatu relatively recently (< 100,000 years ago) (Phillimore, 2006), it is likely that these hosts were susceptible to new parasites and that their immunity gradually co-evolved with the island parasite fauna. This is indicated by the occurrence of six shared parasite lineages between *Z. lateralis* and endemic *Z. flavifrons*, *Z. xanthochrous* and *Z. minutus*. In contrast, lineages that were detected exclusively in *Z. lateralis*, were probably 'spill-over' infections which could have been harboured by other avian hosts or *Zosterops* species.

Within *Z. flavifrons* there was no evidence of higher parasite diversity on larger islands. Some other studies of island fauna have also failed to find a species–area relationship for parasites. In a study of the directly transmitted parasites of *Anolis* lizards, habitat moisture was the most important predictor for helminth distribution rather than island size (Dobson *et al.*, 1992). Similarly, Lindström *et al.* (2004) found no support for the prediction of a species–area relationship for parasites of Darwin's finches; instead, differences in immune response of hosts between islands were a better predictor. Either effects of environmental factors at microhabitat levels or the immune defences of hosts might have influenced the distribution of parasites in *Z. flavifrons* across islands; this requires more detailed assessment than is available here.

The size range of islands used in a study can influence parasite richness patterns and the presence of a widespread host species. The size range of islands used in this study was larger than that used by Fallon *et al.* (2003) in their Lesser Antillean parasite fauna in passerines; however, the overall pattern of parasite richness remained the same even after exclusion of the largest island (mainland New Caledonia) in *Z. lateralis*. The relationship between parasite richness and island size across all *Zosterops* hosts was inflated by the inclusion of the largest island. Although path analysis suggested that inadequate sampling effort had not compromised our estimates of parasite richness, the failure of the accumulation curve to asymptote in mainland New Caledonia ($S_{\mathrm{obs}} = 12$, $S^{*}_{2} = 21.76 \pm 12.10$; Table 1) and also in the endemic *Zosterops* species suggests we may have underestimated the species richness for some islands. Indeed, as a general qualifier here, it should be noted that nonparametric estimators of species richness (Chao2) could significantly underestimate total species richness (O'Dea *et al.*, 2006), which might have caused potential bias in our results and points to the need for much detailed information on other variables (e.g. habitat, elevation etc.) affecting the distribution of parasites across the islands.

The absence of a species–distance relationship for both parasite genera was not consistent with the basic prediction of island biogeography theory. This discrepancy could be due to the dependence of the immigration rates of haematozoan parasites on the presence of both vertebrate and invertebrate hosts. Despite a more or less even distribution of *Z. lateralis* across the islands, the degree of isolation may be accounted for by other factors such as a lack of competent vectors and habitat rather than geographical distance.

Distance–decay relationship

In the avian–mosquito–parasite system studied here, community similarity decayed significantly with distance in *Plasmodium*, mosquitoes and birds; we did not detect this pattern in *Haemoproteus*. The data reveal that the sampled *Plasmodium* lineages and their potential vertebrate and invertebrate hosts are not randomly distributed, but exhibited spatially predictable patterns at different geographical scales. For example, the rate of decay (turnover) in *Plasmodium* was twice that for mosquito species, which in turn was twice that for bird species as a whole (Table 3). The decay of similarity with distance might be caused by either a decrease in environmental similarity with distance (e.g. climatic gradients) or by limits to dispersal and niche width differences among taxa (Nekola & White, 1999; Poulin, 2003). Although *Plasmodium* is ubiquitous and relatively less host-specific than *Haemoproteus* (which ought to provide more opportunities for geographical range expansion), the decline in similarity in *Plasmodium* with distance demonstrates that geographical differentiation exists, which corresponds well with the decline in similarity of mosquito hosts.

One possible explanation for the lack of support for a distance–decay relationship in *Haemoproteus* is its high host fidelity. A limit on the number of locally coexisting parasite species could lead to greater homogeneity in species composition, regardless of distance, by preventing rare parasite species that are not specialized on available hosts from successfully invading a community. Such a pattern has been observed in helminth parasites of fish hosts (Poulin, 2003). Given the high prevalence of *Haemoproteus*, it is possible that *Haemoproteus* lineages are the dominant parasites of *Zosterops* species. In the avifauna of the Lesser Antilles, the presence of particular haematozoan lineages appears to prevent the coexistence of other lineages (Fallon *et al.*, 2003). However, a better understanding of patterns of *Haemoproteus* distribution requires a description of the composition of its primary vectors, i.e. biting midges and simulid flies. If the same fauna of invertebrate hosts occurs on all islands occupied by a given species of avian host, then we may expect distance effects to be diluted by the ecological stability of these habitats. This would result in lower rates of decay among parasite communities.

CONCLUSIONS

Our data illustrate that, like macro-organisms, avian blood parasites are not randomly distributed but rather exhibit spatially predictable patterns. The steep slope of the curve for parasite richness in *Z. lateralis* suggests that the heterogeneity in prevalence and diversity is a result of additional environmental factors as well as the degree of isolation. Distance was not the key determinant of similarity in all parasite communities. A greater rate of distance–decay between islands implies a faster turnover in generalist parasite composition across the islands and hence a steeper species–area relationship. Our findings suggest that further studies of parasite diversity across a range of environmental gradients, transmission mechanisms and host specificities would be valuable.

ACKNOWLEDGEMENTS

We would like to thank E. Bani from the Vanuatu Environment Unit and the chiefs of the Vanuatu islands and D. Paulaud (Director, Environment and Terrestrial Service, South Province, New Caledonia) and J. J. Cassan (Environment Officer, North Province, New Caledonia) for granting permission to sample *Zosterops*. We would like to thank N. I. Mundy, University of Cambridge, for providing laboratory space. This manuscript benefited greatly from the comments of two referees to whom we are very grateful. The avian malaria study (F.I.) was funded by the Marie Curie Fellowship of the European Commission under contract number MIF1-CT-2006-040845. The *Zosterops* project in the Southwest Pacific islands (S.M.C., A.B.P. and R.A.B.) was funded by Natural Environment Research Council (NERC) studentships to A.B.P. and R.A.B. and a NERC fellowship to S.M.C.

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SUPPORTING INFORMATION

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

Appendix S1 DNA extractions, polymerase chain reaction (PCR) and sequencing methods.
Appendix S2 Path model indicating direct and indirect effects of sampling effort on parasite richness.
Appendix S3 Prevalence of *Plasmodium* and *Haemoproteus* by island.
Appendix S4 Parasite lineage accumulation curves by host and islands.
Appendix S5 Species–area relationships of all avian hosts (except seabirds) and mosquito species found in Vanuatu and New Caledonia.

BIOSKETCH

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Author contributions: F.I. and B.C.S. conceived the study; F.I. conducted the molecular work, analysed the data and led the writing; S.M.C, A.B.P. and R.A.B. collected the *Zosterops* samples; S.M.C. and B.C.S. contributed to writing and A.B.P. and R.A.B. commented on the manuscript. B.C.S. and I.P.F.O. initiated the collaboration between the Edward Grey Institute, University of Oxford, and Imperial College London on avian malaria study in the south-west Pacific islands.

Editor: Jon Sadler