Molecular Ecology (2008) 17, 4545–4555 doi: 10.1111/j.1365-294X.2008.03935.x

Avian haematozoan parasites and their associations with mosquitoes across Southwest Pacific Islands

F. ISHTIAQ,* L. GUILLAUMOT,† S. M. CLEGG,‡ A. B. PHILLIMORE,‡§ R. A. BLACK,‡ I. P. F. OWENS,‡§ N. I. MUNDY¶ and B. C. SHELDON*

*Edward Grey Institute, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK, †Entomology Unit, Pasteur Institute of New Caledonia, 9-11 Avenue Paul Doumer BP 61 98845 Noumea cedex, New Caledonia, ‡Division of Biology, Imperial College London, Silwood Park, Ascot, Berkshire SL5 7PY, UK, §NERC Centre for Population Biology, Imperial College London, Silwood Park, Ascot, Berkshire SL5 7PY, UK, ¶Evolutionary Genetics group, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK

Abstract

The degree to which haematozoan parasites can exploit a range of vectors and hosts has both ecological and evolutionary implications for their transmission and biogeography. Here we explore the extent to which closely related mosquito species share the same or closely related haematozoan parasites, and examine the overlap in parasite lineages with those isolated from avian hosts, *Zosterops* species, sampled across the same study sites. Mosquito samples were collected and analysed (14 species, *n* = 804) from four islands in Vanuatu and the main island of New Caledonia. Using polymerase chain reaction, 15.5% (14/90) of pooled mosquito (thoracic) samples showed positive amplifications. Subsequent phylogenetic analysis of the cytochrome *b* gene identified four genetically distinct *Plasmodium* and two *Haemoproteus* lineages from these samples, five of which were identical to parasite lineages (*n* = 21) retrieved from the avian hosts. We found that three *Plasmodium* lineages differing by a maximum of 0.9% sequence divergence were recovered from different species and genera of mosquitoes and two *Haemoproteus* lineages differing by 4.6% sequence divergence were carried by 10 distantly related (11–21% divergent) mosquito species. These data suggest a lack of both cospeciation and invertebrate host conservatism. Without experimental demonstration of the transmission cycle, it is not possible to establish whether these mosquitoes are the biological vectors of isolated parasite lineages, reflecting a limitation of a purely polymerase chain reaction-based approach. Nonetheless, our results raise the possibility of a new transmission pathway and highlight extensive invertebrate host shifts in an insular mosquito–parasite system.

Keywords: *Haemoproteus*, mosquito, *Plasmodium*, Southwest Pacific Islands, sporozoite, *Zosterops*

Received 8 April 2008; revision received 2 August 2008; accepted 12 August 2008

Introduction

Vector-transmitted parasites such as *Plasmodium* and *Haemoproteus* rely on the presence of both an appropriate host and a competent vector (Bennett *et al*. 1974; Apanius *et al*. 2000; Sol *et al*. 2000). The primary vectors for *Haemoproteus* spp. are known to be biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) and louse flies (Diptera: Hippoboscidae) (Atkinson & van Riper 1991).

Correspondence: F. Ishtiaq, Fax: +44 (0) 1865 271168; E-mail: farah.ishtiaq@zoo.ox.ac.uk

Species of avian *Plasmodium* are most commonly transmitted by mosquitoes (Valkiũnas 2005). In avian hosts, *Plasmodium* spp. and *Haemoproteus* spp. are globally distributed and have been detected in a wide range of species (Atkinson & van Riper 1991). With the application of molecular diagnostic techniques, both parasite genera have been shown to exhibit varying degrees of host specificity and modes of transmission (Bensch *et al*. 2000; Ricklefs & Fallon 2002; Waldenström *et al*. 2002; Peréz-Tris & Bensch 2005a). In the past 60 years, numerous laboratory (Reeves *et al*. 1954; Huff 1965) and field studies (LaPointe *et al*. 2005; Woodworth *et al*. 2005; Gager *et al*. 2008) have explored the role of

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd

culicine mosquitoes in the transmission of avian *Plasmodium*. Whilst experimental studies show that anopheline mosquitoes are capable of transmitting avian *Plasmodium* (Mayne 1928; Coggeshall 1941; Haas & Akins 1947), they have not been demonstrated to be natural vectors in the wild. Our knowledge on natural vectors is still expanding, and the possibility remains that new vector groups may be identified. For example, Kleine *et al*. (1987a, b) reported two divergent groups of arthropods that transmit *Plasmodium* among lizards. In addition, a recent study identified louse flies as vectors of a *Haemoproteus* lineage (detected in doves) that is more closely related to *Plasmodium* than other species of *Haemoproteus* (Martinsen *et al*. 2008).

The life cycle of haematozoan parasites involves the sexual phase (sporogony) which occurs in an invertebrate host (vector) and the asexual cycle (schizogony) which takes place in avian hosts. Given that mosquito species vary in their vector competence, ability to support parasite development and environmental preferences (Beier 1998), there are many ways in which mosquitoes potentially alter the relationship between the parasite and the vertebrate host in both evolutionary and ecological contexts. First, dispersal and movement of mosquitoes (e.g. Lum *et al*. 2007) may play an important role in reproductive isolation and gene flow between populations of parasites, as widely dispersing vectors may move parasite strains among host populations, therefore increasing the effective population size of the parasite and reducing losses of genetic diversity (May & Nowak 1994). Second, the dependency of mosquito abundance on environmental conditions could regulate a potential vertebrate host's exposure to parasites; depending on the availability of vectors in dry or wet habitats, parasite prevalence in hosts can differ on a rather small geographical scale (Sol *et al*. 2000; Freeman-Gallant *et al.* 2001; Wood *et al*. 2007). Third, the use of a broad spectrum of blood-feeding vectors facilitates host switching in generalist parasites (Githeko *et al*. 1994). Conversely, parasites with specialized vector associations tend to have a restricted host range (Killick-Kendrick 1978). Comparison of avian host and parasite phylogenies indicates that closely related parasites may be found in distantly related host species, and distantly related parasites can share a single host species (Ricklefs & Fallon 2002; Beadell *et al*. 2004). In contrast, our understanding of the level of vector–parasite specificity and the extent to which closely related mosquito species share the same or closely related haematozoan parasites is very limited. The avian haematozoan literature is biased towards studies that have been conducted on vertebrate hosts and only a handful of studies have compared parasite mitochondrial lineages in both vertebrate and invertebrate hosts (e.g. Gager *et al*. 2008).

In this study, we explore the diversity and phylogeny of haematozoan parasites in wild-caught mosquito species and in a sample of avian hosts, species of the genus *Zosterops* (white-eyes), sampled across the same study sites in Vanuatu and New Caledonia. *Zosterops* samples however, include additional islands within Vanuatu and New Caledonia. Owing to their widespread distribution across the islands of the Southwest Pacific, members of the *Zosterops* complex provide an interesting opportunity to understand host–parasite–mosquito interactions. The mosquito fauna in the South Pacific region and their biogeography are relatively well documented, comprising 27 species (including nine endemics) from New Caledonia and 21 species (including five endemics) from Vanuatu, with seven shared species of mosquito (Laird 1954; Belkin 1962; Maffi 1977). Using DNA sequencing of the cytochrome *b* gene, we examine how the distribution of parasites in vertebrate hosts is associated with their distribution in potential invertebrate hosts. Polymerase chain reaction (PCR)-based approaches alone do not provide definitive evidence of parasite transmission but the detection of shared parasite lineages in vertebrate and dipteran hosts highlights possible pathways of transmission that warrant further investigation. Specifically, we examine (i) the phylogeography and diversity of the parasite lineages found in different mosquito populations, (ii) the extent to which closely related mosquito species share the same or closely related parasites, and (iii) the extent to which parasite lineages are shared between avian hosts and mosquito species in island populations.

Materials and methods

Sample collection and preparation

Female mosquito samples were collected over a period of 6 weeks in March–April 2007, mainly in South (22.26°S, 166.440°E) and North (20.68°S, 164.982°E) Provinces of the main island of New Caledonia, plus four islands in Vanuatu: Efate (17.55°S, 168.294°E), Malekula (16.10°S, 167.416°E), Ambrym (16.28°S, 167.961°E) and Espiritu Santo (15.16°S, 166.948°E) (Fig. 1). Mosquito sampling was generally conducted close to sites where blood samples had been collected from potential avian hosts between February–May in 2004–2006 (Phillimore *et al*. 2008; R. A. Black, personal communication).

In Vanuatu, mosquito sampling was conducted mainly with sweep nets or on landing sites using aspirators. Forest tracks were surveyed morning and evening to identify peak activity times, and swarms of mosquitoes were collected using sweep nets. Mosquito samples were transferred to tubes labelled by date and location, and stored in 70% ethanol for species identification. In New Caledonia, we used eight modified miniature Centre for Disease Control and Prevention (CDC) light traps and dry ice (CO_2; supplied by Air Liquide, Nouméa, France)-baited ultraviolet light traps (www.johnwhock.com) in a range of various habitats: along river banks, in harbour areas, in

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd

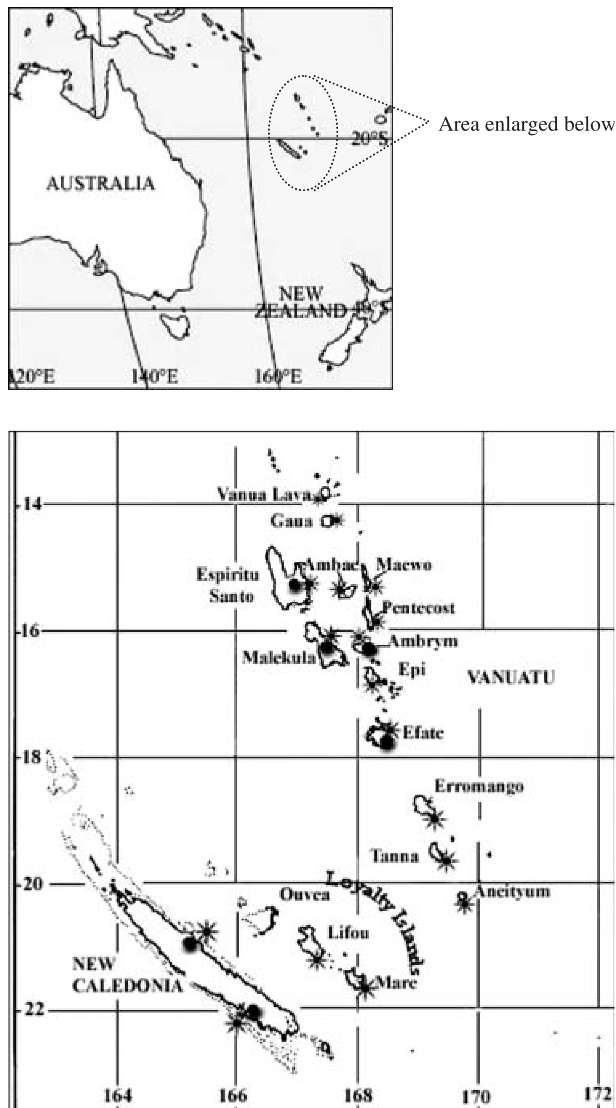

Fig. 1 Map of Vanuatu and New Caledonia archipelagos: sampled islands marked with dots for mosquitoes and stars for *Zosterops* species.

semi-urban areas and in forest. Traps were set out in the evening for 12 h. Mosquitoes were collected from traps in the morning and stored at –20 °C before identification. The species and sex of all mosquito samples was determined using a morphological mosquito-identification key (Belkin 1962). All identifications were made by L.G. at the Pasteur Laboratory in New Caledonia. We collected four species of mosquito ($n = 76$) from Vanuatu: *Anopheles farauti*, *Aedes hebrideus*, *Aedes vexans nocturnus* and *Verrallina lineata,* and 10 species of mosquito ($n = 728$) from North and South Provinces of New Caledonia: *Aedes aegypti*, *Ae. alternans*, *Ae. notoscriptus*, *Ae. vexans*, *Ae. vigilax*, *Coquillettidia xanthogaster*, *Culex annulirostris*, *Cx. (Neoculex) gaufini*, *Cx. quinquefasciatus* and *Cx. sitiens*. All female mosquito samples were sorted by species, date and location and stored as pooled samples in 70% ethanol in labelled tubes for further analysis (see Table 1).

Mosquito dissection. To assess the infection rates and the extent of genetic discrepancy in infective and non-infective stages of parasite lineages, we used PCR techniques to screen the thoracic (including salivary glands) and abdominal parts separately. The infective stage of parasites (sporozoites) is transmitted via the salivary glands of female mosquitoes during blood feeding. The salivary glands are present in the thorax flanking the oesophagus (James & Rossignol 1991) whereas fertilization and oocyst development (non-infective stage) take place in the midgut (abdomen) (Touray *et al*. 1992). Before dissection, mosquitoes from each pooled sample were placed on a Petri dish and the wings and legs removed using two pairs of forceps under a dissecting microscope. Each mosquito was then placed on a microscope slide and divided with a dissecting blade into thorax and abdomen. To avoid potential contamination, we used a new Petri dish, slide and dissecting blade for each pooled sample. Each portion of the body was placed in a separate tube. Forceps were sterilized after each use by dipping in 70% ethanol, immersion in 10% bleach for 10 min and wiped dry. Initially, we analysed each specimen individually using PCR screening to determine the presence of parasites. Owing to low infection rates, the remaining samples were stored in pools of 1 to 20 individuals for PCR screening (Gu 1995). We obtained thoracic ($n = 804$) and abdominal ($n = 455$) parts for each mosquito pool grouped by species, location and stage of blood digestion. Empty abdomens from females ($n = 349$) were not analysed further. We stored thoracic parts of all pooled samples of mosquitoes, whereas abdomens of visibly gravid females ($n = 55$) and those without blood ($n = 374$) were sorted and stored by species and location. Blood-engorged abdomens (visible undigested bloodmeal) ($n = 26$) were stored individually for each specimen.

Extraction of DNA. DNA extractions were performed using the QIAGEN Mini Prep Kit with a procedure specially optimized for mosquito tissues (Pilchart *et al*. 2006). Single mosquitoes and pooled samples (see Table 1) were dried for a minimum of 2 h at 70 °C, before being carefully crushed with a sterile pestle tissue grinder (Fisher Scientific) in 1.5 mL microcentrifuge tubes. Each extraction included a negative control, where the extraction process was repeated without adding mosquito tissue. No product was amplified from extraction controls.

Molecular analysis–parasite screening

Samples were screened with primers F2/R2 (Beadell *et al*. 2004), designed to amplify small fragments (91 bp) of

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd

Table 1 Avian haematozoan parasite prevalence in female mosquito species collected in Vanuatu and New Caledonia

Mosquito species	Sampling locations	Thorax						Abdomen					
		Total *N*	Pool size	Proportion of positive pools (percentage)	MLE	MIR	IR	Total	Pool size	Proportion of positive pools (percentage)	MLE	MIR	IR
Vanuatu													
Anopheles farauti	MAL	2	1,1	0	0	0	100	2	1,1	0	0	0	100
Aedes hebrideus	MAL, EF, AMB	14	1–8	33 (1/3)	128.31 (5.62, 524.62)	83	33 (1/3)	14	1–8	33 (1/3)	128.31 (5.62, 524.62)	83	33 (1/3)
Aedes vexans nocturnus	MAL	1	1	0	0	0	0	1	1	0	0	0	0
Verrallina lineata	EF, AMB, SAN	59	1–11	12.5 (1/8)	18.90 (1.06, 84.35)	17.86	50 (1/2)	60	1–11	22 (2/9)	40 (6.90, 122.22)	34.48	0
New Caledonia													
Aedes aegypti	SP	3	1, 2	100 (1/1)	NA	500	0	3	1, 2	100 (1/1)	NA	500	0
Aedes alternans	SP	4	1	100 (1/1)	NA	250	0	4	1	100 (1/1)	NA	250	0
Aedes notoscriptus	SP, NP	11	1–6	0 (0/2)	0	0	0	11	1–6	0 (0/2)	0	0	100 (1/1)*
Aedes vexans	SP	2	1	100	NA	500	0	2	1	100	NA	500	0
Aedes vigilax	SP, NP	179	5–16	12.5 (2/16)	11.67 (2.08, 36.80)	11.24	0	115	1–16	15.3 (2/13)	19.11 (3.34, 60.07)	17.54	0
Coquillettidia xanthogaster	SP	77	2–10	10 (1/10)	5.83 (0.34, 27)	5.81	0	53	1–10	10 (1/10)	19.42 (1.14, 83.63)	19.23	0
Culex annulirostris	SP	28	4–10	75 (3/4)	206.65 (45.82, 506.65)	107.14	0	13	4–9	100 (2/2)	NA	153.85	0
Culex gaufini	SP	31	5–10	0 (0/4)	0	0	0	6	1	0	0	0	0
Culex quinquefasciatus	SP, NP	263	1–20	8 (2/25)	7.75 (1.40, 24.35)	7.66	0	101	1–20	23 (3/13)	31.27 (8.61, 76.13)	30.3	0
Culex sitiens	SP	130	2–20	20 (2/10)	15.50 (2.90, 45.43)	15.38	0	70	2–20	28 (2/7)	29 (5.60, 80)	28.57	0
		804						455					

MAL, Malekula; EF, Efate; AMB, Ambrym; SAN, Espiritu Santo; SP, South Province; NP, North Province. Pool size refers to the number of individuals combined by species/date/location into a single sample of DNA extraction. MLE, maximum likelihood estimation (per 1000 mosquitoes) (upper, lower 95% confidence limits) based on pool size; MIR, minimal infection rate (per 1000 mosquitoes); IR, infection rate (percentage) based on number of individually tested mosquitoes; *only abdominal part showed infection.

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd

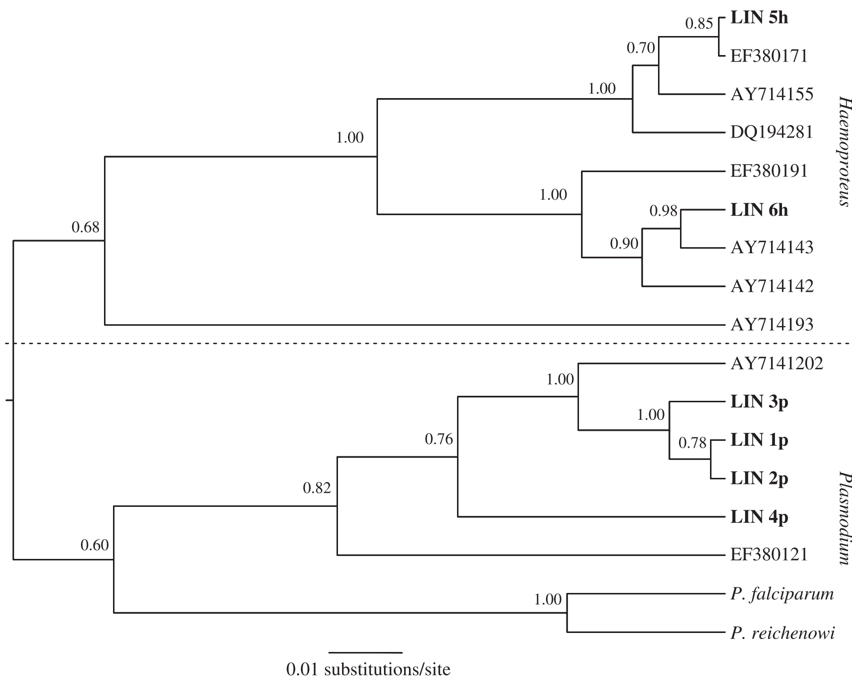

Fig. 2 Phylogeny of haematozoan lineages based on 351 bp of the cytochrome *b* gene. Maximum clade credibility tree from Bayesian relaxed clock analysis. Support values indicate posterior clade probabilities. Lineages in bold were retrieved from mosquito samples in this study. Known parasite sequences from GenBank are included to confirm lineage identification as *Haemoproteus* or *Plasmodium*. GenBank numbers indicate avian host and geographical origin of lineages. EF380171 = *Zosterops palpebrosus* (India); AY714155 = *Zosterops fuscicapillus* (Papua New Guinea); DQ194281 = *Acridotheres tristis* (India); EF380191 = *Prinia socialis* (India); AY714143 = *Zosterops fuscicapillus* (Papua New Guinea); AY714142 = *Crateroscelis murina* (Papua New Guinea); AY714193 = *Macropygia amboinensis* (lineage in dove) Papua New Guinea); AY7141202 = *Pitta versicolour* (Papua New Guinea); EF380121 = *Estrilda amandava* (India).

haematozoan parasite mitochondrial DNA (cytochrome *b* gene). For those samples that were positive for parasites based on the above test, we amplified two larger fragments using primers Fifi/4292 rw2 (351 bp) (Ishtiaq *et al.* 2006) and F3/R3 (271 bp) (Beadell *et al.* 2004). All PCRs included a negative control that contained all PCR reagents but no DNA. We purified the largest of the PCR products (351 bp or 271 bp) available using QIAquick kits (QIAGEN) and sequenced the fragment on an ABI 3100 Sequencer on both strands using the PCR primers (Applied Biosystems, Inc.). Sequences were assembled, aligned and edited using Sequencher version 4.1. The inclusion of lineages from previous studies of avian malaria parasites with known morphology (Perkins & Schall 2002; Beadell *et al.* 2004; Ishtiaq *et al.* 2007) allowed us to define *Plasmodium* and *Haemoproteus* clades. (see Fig. 2). All sequences were deposited in GenBank (Accession FJ025891–FJ025896).

Mosquito DNA amplification. To ensure that failure to detect a parasite was not due to poor DNA extractions from the samples as a whole, we amplified a small fragment of mosquito cytochrome *b* DNA using universal primers Cytb-2RC and Cytb-WOW (268 bp) (Dumbacher *et al.* 2003). These PCRs were successful in amplification of mosquito DNA in all cases. Mosquito samples with parasite amplifications were sequenced in both forward and reverse directions. There were no sequences from the species of interest available for comparison in GenBank. However, we determined whether each distinct cytochrome *b* sequence corresponded to a distinct morphological mosquito species. All sequences were deposited in GenBank (Accession FJ025877–FJ025890).

Statistical analyses of prevalence

We used a maximum likelihood estimation (MLE, Gu *et al.* 2003) method using the PooledInfRate program (www.cdc.gov) to calculate the proportion of infected individuals in pooled samples. Heterogeneity in parasite prevalence was then assessed using chi-squared tests in SPSS version 9.1.

Phylogenetic analysis

We estimated the phylogenetic relationships among parasite lineages using samples for which we had at least 271 or 351 bp of cytochrome *b* sequence. We used an Akaike's information criterion-based approach in Modeltest, version 3.7 (Posada & Crandall 1998), to determine the most appropriate evolutionary model (GTR + *G*). Phylogenetic reconstruction was implemented in BEAST version 1.4.7 (Drummond & Rambaut 2007) using the Markov chain Monte Carlo (MCMC) algorithm. We present a maximum clade credibility tree using a relaxed clock approach (Drummond *et al.* 2006) with a mean substitution rate set at 1% per lineage per million years. Rates of substitution were drawn from a log-normal distribution and Yule prior was used for branching rates. We conducted two runs for 10 million generations, with sampling conducted every 1000 generations. *Tracer* (Rambaut &

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd

Drummond 2003) was used to assess convergence, whether two chains were mixing, and whether the estimated sample (ESS) for each parameter was of sufficient size (ESS > 200) to obtain good parameter estimates. Two million generations were discarded as burn-in from each run, leaving a posterior distribution of 16 000 trees. The phylogenetic tree was rooted using published sequences of two mammalian *Plasmodium* parasites *P. reichenowi* (AF069610) and *P. falciparum* (AF069605) as outgroups (see Perkins & Schall 2002). We used MEGA 4.0 (Kumar *et al*. 2004) to estimate pairwise genetic distances among parasite lineages and among mosquito species.

To examine the extent to which the vertebrate host range of parasites on each island was matched by the distribution of their invertebrate hosts, we constructed a phylogenetic tree of parasite lineages obtained from the mosquito species ($n = 6$ lineages) with those obtained from *Zosterops* species ($n = 21$ lineages) and recorded the identical sequences. Samples from *Zosterops* species in Vanuatu and New Caledonia (total $n = 692$) were infected with 21 distinct parasite lineages, consisting of 14 *Plasmodium* and seven *Haemoproteus* (F. Ishtiaq *et al*. in preparation).

Results

Prevalence of parasites

Using PCR and parasite-specific primers, we screened 1259 (804 thorax and 455 abdomen; see methods) parts of 14 mosquito species combined into 150 pools of variable sizes from four islands in Vanuatu and mainland New Caledonia (Fig. 1). Positive PCR amplifications from abdominal and thoracic parts indicated the presence of parasites (henceforth referred to as an infection). The parasite prevalence (infection rate) calculated by MLE showed no significant difference between thoracic parts (assumed to represent sporozoite) (19.73 per 1000 (95% CI 11.68–31.01) and abdominal parts (assumed to represent oocyst) (39.25 per 1000 (95% CI 23.85–60.02; $\chi^2 = 0.98$, $P = 0.32$; see Table 1). The parasite prevalence in individually tested mosquito samples was 38.46% (5/13) in thoracic parts and 25% (3/12) in abdominal parts. None of the individually analysed engorged abdominal samples showed evidence of parasite DNA. Owing to low sample sizes of certain mosquito species, we did not test for differences in the parasite prevalence among mosquito species. Table 1 provides a detailed account of infections by species.

From Vanuatu, a total of 76 thoracic samples of mosquitoes were screened in a total of 11 pools, of which six were positive for parasite DNA. In samples from Malekula, 4/5 (80%) of mosquito pools showed positive for parasite DNA, of which three were *Haemoproteus* spp., and one *Plasmodium* spp. and in those from Efate, 2/6 (33%) were positive for *Haemoproteus* spp. Mosquitoes sampled on Ambrym and Espiritu Santo showed no evidence of parasite DNA. While there was no significant difference in parasite prevalence between these islands ($\chi^2_3 = 4.60$, $P = 0.20$), no strong conclusions can be drawn owing to a lack of statistical power.

From New Caledonia, a total of 728 individuals were screened across 79 pools, of which 14 were positive for haematozoan DNA. None of the pools ($n = 4$) from the North province showed evidence of parasite DNA. In the South Province, however, 11/75 (14%) were positive for *Haemoproteus* spp. and 3/75 (4%) for *Plasmodium* spp.

Diversity and distribution of parasite lineages

We sequenced parasite mitochondrial DNA (mtDNA) from 23 pooled and nine individual mosquito samples that were positive for haematozoan DNA. In abdominal pools ($n = 9$), two pools showed mixed lineages, while their corresponding thoracic pools were infected with single lineages. In one case, a lineage retrieved from an abdominal sample of an individual *Aedes notoscriptus* was not found in the corresponding thoracic part. In the remaining cases, the lineages amplified from abdominal pools were identical to their corresponding thoracic pools. Abdominal lineages represent the non-infective stage of the parasite or parasite picked up in bloodmeals; these lineages were not used in the analysis except for the case mentioned above where a distinct lineage was retrieved from a single abdomen. Among thoracic pools ($n = 14$), two cases had sequences with double peaks in the chromatogram indicating double lineages but no parasite in the corresponding abdominal parts. One particular lineage dominated the sequence in both cases, and we could thus determine the 'main' lineage in the samples. Although techniques to resolve double lineages exist (Peréz-Tris & Bensch 2005b), these were not used as there was insufficient DNA template. Identifying the 'main' lineages from double infected pooled samples has however, previously been used to build parasite phylogenies (Gager *et al*. 2008; Hellgren *et al*. 2008). Our dominant lineage approach might have biased the data towards the most prevalent group of parasites in two cases. In the remaining pools ($n = 12$) and individual cases ($n = 8$), clean sequences were retrieved. Among readable sequences, we identified four genetically distinct *Plasmodium* (LIN 1p to LIN 4p) differing from each other by 0.4% to 4.1% and two *Haemoproteus* (LIN 5h and LIN 6h) lineages differing by 4.6% of sequence divergence (Fig. 2). LIN 3p was retrieved from the one abdominal sample included in the analyses and was not found in any thoracic part. We identified one *Plasmodium* and two *Haemoproteus* lineages and three *Plasmodium* and two *Haemoproteus* lineages in Vanuatu and New Caledonia, respectively. None of the *Plasmodium* lineages

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd

Table 2 Distribution of *Plasmodium* and *Haemoproteus* lineages isolated from thoracic parts of mosquito species and their overlap with lineages detected in *Zosterops* species sampled at the same study sites. Mosquito species are arranged based on their phylogenetic relationships. Parasite lineage names refer to Fig. 2. Numbers in the cells indicate lineage frequency and corresponds to particular mosquito and bird species

		Parasite lineages						Samples screened
	Sampling location*	LIN 1p	LIN 2p	LIN 3p	LIN 4p	LIN 5h	LIN 6h	
Mosquito species								
Anopheles farauti	MAL					1	1	2
Aedes hebrideus	MAL, EF, AMB		1				1	14
Aedes alternans	SP						1	4
Aedes vexans	SP						1	2
Aedes vexans nocturnus	MAL							1
Verrallina lineata	EF, AMB, SAN						2	59
Aedes notoscriptus	NP, SP			1				11
Aedes aegypti	SP						1	3
Culex sitiens	SP	1					1	130
Culex annulirostris	SP	1			1		1	28
Culex quinquefasciatus	NP, SP					1	3	263
Aedes vigilax	NP, SP					1	1	179
Coquillettidia xanthogaster	SP					1		77
Culex gaufini	SP							31
Zosterops species								
Z. lateralis	MAL, SAN, NP, SP LIF		12			4	7	269
Z. flavifrons	MAL, SAN, MAE, TAN					4	2	338
Z. xanthochrous	NP, SP, MAR	1		1			6	60
Z. minutus	LIF						1	25

*Vanuatu: MAL, Malekula; EF, Efate; AMB, Ambrym; SAN, Espiritu Santo; MAE, Maewo; TAN, Tanna. New Caledonia: NP, North Province; SP, South Province; MAR, Mare; LIF, Lifou.

were shared between the two regions. Both *Haemoproteus* lineages were however, shared between Vanuatu and New Caledonia. *Plasmodium* lineages were detected at substantially lower frequencies than *Haemoproteus* lineages (Table 2).

Mitochondrial DNA (cyt *b* gene) sequences from mosquitoes showed distinct lineages which corresponded perfectly with the morphological identity of the mosquito species. Within-genus nucleotide divergence ranged from 3.6% to 15.2% in *Aedes* and from 0.5% to 15.8% in *Culex*. Inter-genus DNA sequence divergence was more pronounced, ranging between 7.5% and 21.7%. There were cases where (i) a single parasite lineage was identified in multiple mosquito species, and (ii) multiple parasite lineages were found within a single mosquito species. *Plasmodium* lineage LIN 1p was detected in both *Culex sitiens* and *Culex annulirostris* from New Caledonia that were separated by 10.3% mtDNA sequence divergence. LIN 1p clustered with two other *Plasmodium* lineages, LIN 2p (0.4% sequence divergence from LIN 1p) and LIN 3p (0.9% sequence divergence from LIN 1p). These closely related lineages were detected in two very divergent species of mosquito (10–19% sequence divergence). LIN 6h isolated from 10/14 of the mosquito species accounted for nearly 60% of all infections, followed by LIN 5h for 29% infections (Table 2).

In Table 2, we show the distribution of parasite lineages found in the mosquito species and their overlap with parasite lineages detected in avian hosts (*Zosterops* spp.). Five of the six lineages found in mosquito species were detected from a total of 38 positive *Zosterops* samples across these islands. Interestingly, the frequency of *Haemoproteus* lineages showed no significant difference between avian hosts and mosquito species (LIN 6h; $\chi^2 = 0.31$, $P = 0.55$ and LIN 5h; $\chi^2 = 0.12$, $P = 0.72$). Furthermore, most of the avian hosts and mosquitoes sampled on islands shared the same parasite lineages. LIN 4p (matching *Plasmodium juxtanucleare*, GenBank no. AB602893) was the only lineage that was not shared with any lineage isolated from the *Zosterops* spp. Among 21 parasite lineages found in the *Zosterops* species, 15 were not detected in the mosquito species.

Discussion

The degree to which haematozoan parasites can exploit a range of vectors and hosts has both ecological and evolutionary implications for their transmission and

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd

biogeography. To understand these interactions in a highly complex system, all three taxonomic components (parasite, invertebrate host and vertebrate host) need to be considered. Molecular screening of parasites in mosquitoes and avian hosts across islands in the Southwest Pacific revealed: (i) a broad spectrum of mosquito species could potentially harbour closely related or identical parasite lineages; (ii) the distribution of parasite lineages across the Vanuatu and New Caledonia archipelagos show corresponding patterns and associations in vertebrate and invertebrate hosts; and (iii) culicine and anopheline mosquitoes could unexpectedly harbour *Haemoproteus* spp., in addition to *Plasmodium* lineages. However, their potential role in transmission of these parasite lineages, if any, needs to be confirmed by other methods.

Phylogeography and diversity of parasite lineages in mosquito species

The distribution of parasite lineages across mosquito species and geographical locations indicates the existence of an assemblage of common parasite lineages in the region that potentially infect multiple, and often genetically divergent, host species. For *Plasmodium*, this was reflected in the detection of three closely related parasite lineages in four divergent mosquito species. For *Haemoproteus* lineages, the effect was even more pronounced, with one lineage found in 10 divergent mosquito species across the two archipelagos. These findings suggest that these lineages have low host specificity, and therefore the opportunities for invertebrate host shifts may be high in this system.

There was considerable heterogeneity in the parasite prevalence in mosquito species which might be related to the abundance and distribution of respective mosquito populations at each location, or to the variation in sampling techniques. Despite this limitation, detection of *Haemoproteus* spp. in *Anopheles farauti* and other mosquito species in Vanuatu and New Caledonia suggests that the human malaria vector *An. farauti* could unexpectedly carry distantly related avian haematozoan parasites (i.e. *Haemoproteus* spp.). This further illustrates the broad parasite assemblages across multiple mosquito species as well as within mosquito species in this system. The absence of *An. farauti* or any other anopheline species is conspicuous in New Caledonia and explains why this is a human malaria-free region. However, the distribution of haematozoan parasites seems not to be restricted by the lack of shared mosquito species between two regions (Table 2). Given the similarity in mosquito fauna, vertebrate hosts, habitat and climate of the two regions, it is not surprising that Vanuatu and New Caledonia shared *Haemoproteus* lineages. The movement of avian hosts (Bensch *et al*. 2000; Ricklefs & Fallon 2002; Waldenström *et al*. 2002) and human transport (Lum *et al*. 2007) for salt-water tolerant mosquito species (Sweeney 1987) can form an effective bridge for parasites and spread them widely across the regions.

Diversity and distribution of parasite lineages in avian hosts

A comparison of the parasite lineages detected in mosquitoes with those detected in avian hosts, species in the genus *Zosterops* revealed five lineages that were shared between vertebrate and invertebrate hosts and found at the same sites. Given that some lineages could be transmitted by multiple mosquito species, the prevalence of generalist lineages would be expected to be higher across different islands than that of mosquito-specific lineages. The occurrence of generalist *Haemoproteus* lineages was relatively high in both vertebrate and invertebrate hosts which suggest that mosquito–parasite associations facilitate host switching and a broad distribution at different geographical scales of these island systems. The low frequencies of two *Plasmodium* lineages (LIN 2p and LIN 3p) isolated from the endemic mosquito species in Vanuatu and New Caledonia, respectively (Table 2) suggest that these lineages may have a restricted range owing to their invertebrate host specificity.

There were 15 parasite lineages found in avian hosts that were not detected in the mosquito species sampled as part of this study. The large number of missing parasite lineages in mosquitoes is likely due to the limited sampling of the known vector groups. A contributing factor may also be that some islands sampled for birds were not sampled for mosquitoes. Furthermore, different timing of vertebrate and invertebrate samplings could have resulted in discrepancy in prevalence and distribution of certain parasite lineages. Year-to-year temporal fluctuations in parasite lineages (Wood *et al*. 2007) as well as seasonal effects (Bensch & Åkesson 2003; Cosgrove *et al*. 2008) have been detected in some studies. In addition, vector competence can be highly variable within a species and colonization of avian hosts could bring novel parasites to the islands. Therefore, additional geographical and temporal sampling of mosquitoes would increase the likelihood of finding missing lineages in the same, or other arthropods.

Avian *Plasmodium* spp. exploit culicine mosquitoes for transmission to vertebrate hosts (Valkiũnas 2005). However, if any of the mosquito species were refractory (parasites are unable to develop into infectious sporozoites) to the amplified *Plasmodium* spp., then avian hosts would not be exposed to them. It seems likely that at least some of the detected *Plasmodium* lineages are capable of achieving a sporozoite phase, and infecting a vertebrate host, and indeed, three of the four *Plasmodium* lineages found in mosquitoes were retrieved from *Zosterops*. The existence of four distinct *Plasmodium* lineages found in only three mosquito species suggests that these parasite lineages have

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd

undergone a phase of reproductive isolation within the invertebrate hosts and could possibly be considered reproductively isolated units. Studies on avian *Plasmodium* cytochrome *b* sequences from vertebrate hosts have shown that lineages with genetic distances as little as 1% exhibit no nuclear gene recombination, indicating reproductive isolation (Bensch *et al*. 2004). Two of the *Plasmodium* lineages identified in this study retrieved from the same mosquito species (LIN 1p and LIN 4p in *Culex annulirostris*) differed by 4.1% sequence divergence. The sharing of the same mosquito species raises doubts as to whether the lineages represent the same species, despite high sequence divergence. There is also the suggestion that the two lineages may have different avian hosts as only LIN 1p was retrieved from *Zosterops*. LIN 4p was found to be identical to published sequences of *Plasmodium juxtanucleare*, a widely distributed parasite that primarily infects chickens (*Gallus gallus domesticus*) (Kissinger *et al*. 2002), although we are not aware of any reports of this *Plasmodium* spp. infecting chickens in New Caledonia or Vanuatu. Interestingly, one of our sampled mosquito species, *Culex sitiens* has been identified as a natural vector of *P. juxtanucleare* (Bennett *et al*. 1966). The degree to which different *Plasmodium* lineages detected in mosquitoes in this study represent reproductively isolated lineages requires further sampling to determine their vector specificity.

Detection of Haemoproteus *in mosquito species*

Determination of the proportion of mosquitoes in natural populations that develop the oocyst and sporozoite stage is useful information to help in identifying the bottlenecks of parasite development. Based on the analysis of thoracic and abdominal samples, our findings highlight the possibility that these parasite species could achieve the oocyst or sporozoite stage in sampled mosquito species (especially in gravid females). One intriguing finding of this study was that PCR screening detected *Haemoproteus* parasites in culicine and anopheline mosquito species. There is no previous evidence of *Haemoproteus* transmission by mosquito species, and therefore, it would be surprising and controversial if this represents a new type of vector for this parasite. Several alternative explanations may account for the presence of *Haemoproteus* in our mosquito samples. First is the possibility that we amplified parasite residue in the oesophagus or mouthparts picked up during bloodmeals on infected vertebrates. While this is possible, it seems unlikely that blood would still be present in the oesophagus but absent from the abdomen of gravid females (none of the blood engorged females showed evidence of parasite DNA in either body part). Second is the possibility of the presence of non-infectious sporozoites. The infectivity of the sporozoites can be assessed only via inoculations of suitable hosts or development of stage-specific molecular markers (ribosomal RNA, T. McCutchan, NIH, USA, personal communication). Our finding highlights the limitation of PCR-based approach and the need for more sporozoite sampling in wild-caught mosquito species, supplemented with experimental demonstration.

Conclusions

Our multitier approach helps to understand the patterns of parasite diversity and distribution in vertebrate and invertebrate hosts across Southwest Pacific Islands and that associations between host–parasites are possibly influenced by mosquito–parasite interactions. Our study highlights extensive invertebrate host sharing in this parasite–mosquito system and raises the possibility of a new transmission pathway. An experimental demonstration is required to confirm if these mosquito species are susceptible or refractory to the detected parasite lineages. Future research could involve intensive arthropod sampling to identify patterns of parasite endemism, diversity and vector competence in the South Pacific region.

Acknowledgements

We are grateful to E. Bani from the Vanuatu Environment Unit and the chiefs of Malekula, Santo, and Ambrym and to D. Palaud (Director, Environment and Terrestrial Service, South Province, NC) and J.J. Cassan (Environment officer, North Province, NC) for granting permission to catch mosquitoes. We also thank Stephen Totterman (Vanbirds) and other members of Wantok Environment Centre, N. Barré (SCO), C. Rutz, L. Bluff (NC Crow team, Oxford) for providing information on island logistics. We particularly thank Sosiasi Kilama (Pasteur Institute) and C. Bowden for their assistance in the field. Our posthumous thanks to H. Blaffart (CI) for his invaluable help in North Province (NC). We thank J. Beadell, T. Jenkins, O. Hellgren, K. Durrant and three anonymous reviewers for insightful comments on the manuscript. The study on avian haematozoa (FI) was funded by the Marie-Curie Fellowship of the European Commission under contract number MIF1-CT-2006-040845. The *Zosterops* project in the South-west Pacific islands (S.M.C., A.B.P. and R.A.B.) was funded by the Natural Environment Research Council (NERC) studentships to A.B.P. and R.A.B. and an NERC fellowship to S.M.C.

References

Apanius V, Yorinks N, Bermingham E, Ricklefs RE (2000) Island and taxon effects in parasitism and resistance of Lesser Antillean birds. *Ecology*, **81**, 1959–1969.

Atkinson CT, van Riper C III (1991) Pathogenicity and epizootiology of avian haematozoa: *Plasmodium*, *Leucocytozoon*, and *Haemoproteus*. In: *Bird-Parasite Interactions: Ecology, Evolution and Behaviour* (eds Loye JE, Zuk M.), pp. 19–48. Oxford University Press, New York.

Beadell JS, Gering E, Austin J *et al*. (2004) Prevalence and differential host-specificity of two avian blood parasite genera in the Australo-Papuan region. *Molecular Ecology*, **13**, 3829–3844.

Beier CJ (1998) Malaria parasite development in mosquitoes. *Annual Review of Entomology*, **43**, 519–543.

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd

Belkin JN (1962) *The Mosquitoes of the South Pacific (Diptera, Culicidae)*, Vol. I and II. University of California Press, Berkeley, California.

Bennett GF, Warren M, Cheong WH (1966) Biology of the Malaysian strain of *Plasmodium juxtanucleare* Versiani and Gomes, 1941. II. The sporogonic stages in *Culex* (*Culex*) *sitiens* Wiedmann. *Journal of Parasitology*, **52**, 647–652.

Bennett GF, Campbell AG, Cameron M (1974) Hematozoa of passeriform birds from insular Newfoundland. *Canadian Journal of Zoology*, **52**, 765–772.

Bensch S, Åkesson S (2003) Temporal and spatial variation of haematozoans in Scandinavian willow warblers. *Journal of Parasitology*, **89**, 388–391.

Bensch S, Stjernman M, Hasselquist D *et al*. (2000) Host specificity in avian blood parasites: a study of *Plasmodium and Haemoproteus* mitochondrial DNA amplified from birds. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 1583–1589.

Bensch S, Peréz-Tris J, Waldenström J, Hellgren O (2004) Linkage between nuclear and mitochondrial DNA sequences in avian malaria parasites: multiple cases of cryptic speciation? *Evolution*, **58**, 1617–1621.

Coggeshall LT (1941) Infection of *Anopheles quadrimaculatus* with *Plasmodium cynomolgi*, a monkey malaria parasite, and with *Plasmodium lophurae*, an avian malaria parasite. *American Journal of Tropical Medicine*, **21**, 525–530.

Cosgrove CL, Wood MJ, Day K *et al*. (2008) Seasonal variation in *Plasmodium* prevalence in a population of blue tits *Cyanistes caeruleus*. *Journal of Animal Ecology*, **77**, 540–548.

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A (2006) Relaxed phylogenetics and dating with confidence. *Public Library of Science, Biology*, **4**, e88.

Dumbacher JP, Pratt TK, Fleischer RC (2003) Phylogeny of the owlet-nightjars (Aves: Aegothelidae) based on mitochondrial DNA sequence. *Molecular Phylogenetics and Evolution*, **29**, 540–549.

Freeman-Gallant CR, O'Connor KD, Breuer ME (2001) Sexual selection and the geography of *Plasmodium* infection in Savannah sparrows (*Passerculus sandwichensis*). *Oecologia*, **127**, 517–521.

Gager AB, Loaiza RJD, Dearbon DC *et al*. (2008) Do mosquitoes filter the access of *Plasmodium* cytochrome *b* lineages to an avian host? *Molecular Ecology*, doi: 10.1111/j.1365-294X. 2008.03764.x.

Githeko AK, Service MW, Mbogo CM *et al*. (1994) Origin of blood meals in indoor and outdoor resting malaria vectors in western Kenya. *Acta Tropica*, **58**, 307–316.

Gu W (1995) Estimating sporozoites rate by examining pooled samples of mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **89**, 359–360.

Gu W, Lampman R, Novak RJ (2003) Problems in estimating mosquito infection rate using minimum infection rate. *Journal of Medical Entomology*, **40**, 596–596.

Haas VH, Akins H (1947) Transmission of *Plasmodium gallinaceum* by *Anopheles quadrimaculatus*. *Journal of the National Malaria Society*, **6**, 244–245.

Hellgren O, Bensch S, Malmqvist B (2008) Bird hosts, parasites and their vectors-associations uncovered by molecular analyses of blackfly blood meals. *Molecular Ecology*, **17**, 1605–1613.

Huff CG (1965) Susceptibility of mosquitoes to avian malaria. *Experimental Parasitology*, **16**, 107–132.

Ishtiaq F, Beadell JS, Baker AJ *et al*. (2006) Prevalence and evolutionary genetics of haematozoan parasites in native versus introduced populations of common myna *Acridotheres tristis*. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 587–594.

Ishtiaq F, Gering E, Peirce M *et al*. (2007) Prevalence and diversity of avian haematozoan parasites in Asia: a regional survey. *Journal of Wildlife Diseases*, **43**, 382–398.

James AA, Rossignol PA (1991) Mosquito salivary glands: parasitological and molecular aspects. *Parasitology Today*, **1**, 267–271.

Killick-Kendrick R (1978) Taxonomy, zoogeography and evolution. In: *Rodent Malaria* (eds Killick-Kendrick R, Peters W), pp. 1–52. Academic Press, London.

Kissinger JC, Souza PCA, Soares CO *et al*. (2002) Molecular phylogenetic analysis of the avian malarial parasite *Plasmodium (Novyella) juxtanucleare. Journal of Parasitology*, **88**, 769–773.

Kleine TA, Young DG, Telfords SR Jr (1987a) Vector incrimination and experimental transmission of *Plasmodium floridense* by bites of infected *Culex (Melanoconion) erraticus*. *Journal of the American Mosquito Control Association*, **3**, 165–l75.

Kleine TA, Young DG, Telfords SR Jr. *et al*. (1987b) Experimental transmission of *Plasmodium mexicanum* by bites of infected *Luczomyia vexator* (Diptera: Psycodidae). *Journal of the American Mosquito Control Association*, **3**, 154–164.

Kumar S, Tamura K, Nei M (2004) MEGA 4.0: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics*, **5**, 150–163.

Laird M (1954) A mosquito survey in New Caledonia and the Belep Islands, with new locality records for two species of *Culex*. *Bulletin of Entomological Research*, **45**, 285–293.

LaPointe DA, Goff ML, Atkinson CT (2005) Comparative susceptibility of introduced forest-dwelling mosquitoes in Hawai'i to avian malaria, *Plasmodium relictum. Journal of Parasitology*, **91**, 843–849.

Lum JK, Kaneko A, Taleo G *et al*. (2007) Genetic diversity and gene flow of humans, *Plasmodium falciparum*, and *Anopheles farauti s.s.* of Vanuatu: inferred malaria dispersal and implications for malaria control. *Acta Tropica*, **103**, 102–107.

Maffi M (1977) Contribution to the knowledge of the mosquito fauna of the New Hebrides islands group proper (Diptera: Culicidae). *Rivista di Parassitologia*, **38**, 194–215.

Martinsen SL, Perkins SL, Schall JJ (2008) A three-genome phylogeny of malaria parasites (*Plasmodium* and closely related genera): evolution of life-history traits and host switches. *Molecular Phylogenetics and Evolution*, **47**, 261–273.

May RM, Nowak MA (1994) Superinfection, metapopulation dynamics, and the evolution of diversity. *Journal of Theoretical Biology*, **170**, 95–114.

Mayne B (1928) Anopheline mosquitoes as hosts for parasites of bird malaria. *Indian Journal of Medical Research*, **16**, 557–558.

Peréz-Tris J, Bensch S (2005a) Dispersal increases local transmission of avian malarial parasites. *Ecology Letters*, **8**, 838–845.

Peréz-Tris J, Bensch S (2005b) Diagnosing genetically diverse avian malarial infections using mixed-sequence analysis and TA-cloning. *Parasitology*, **131**, 15–23.

Perkins SL, Schall JJ (2002) A molecular phylogeny of malarial parasites recovered from cytochrome *b* gene sequences. *Journal of Parasitology*, **88**, 972–978.

Phillimore AB, Owens IPF, Black RA *et al*. (2008) Complex patterns of genetic and phenotypic divergence in an island bird and the consequences for delimiting conservation units. *Molecular Ecology*, **17**, 2839–2853.

Pilchart C, Sechan Y, Davies N, Legrand A (2006) PCR and dissection as tools to monitor filarial infection of *Aedes polynesiensis* mosquitoes in French Polynesia. *Filaria Journal*, **5**, 2 doi:10.1186/ 1475-2883-5-2.

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Rambaut A, Drummond AJ (2003) *Tracer version 1.3*. Available from URL: http://evolve.zoo.ox.ac.uk/.

Reeves WC, Herold RC, Rosen L, Brookman B, Hammon WM (1954) Studies on avian malaria in vectors and hosts of encephalitis in Kern County, California.2. Infections in mosquito vectors. *American Journal of Tropical Medicine and Hygiene*, **3**, 696–703.

Ricklefs RE, Fallon SM (2002) Diversification and host switching in avian malaria parasites. *Proceedings of the Royal Society B: Biological Sciences*, **269**, 885–892.

Sol D, Jovani R, Torres J (2000) Geographical variation in blood parasites in feral pigeons: the role of vectors. *Ecography*, **23**, 307–314.

Sweeney AW (1987) Larval salinity tolerances of the sibling species of *Anopheles farauti*. *Journal of American Mosquito Control Association*, **3**, 589–592.

Touray MG, Warburg A, Laughinghouse A *et al*. (1992) Developmentally regulated infectivity of malaria sporozoites for mosquito salivary glands and vertebrate host. *Journal of Experimental Medicine*, **175**, 1607–1612.

Valkiũnas G (2005) *Avian malaria parasites and other haemosporidia*. CRC Press, Boca Raton, Florida.

Waldenström J, Bensch S, Kiboi S *et al*. (2002) Cross-species infection of blood parasites between resident and migratory songbirds in Africa. *Molecular Ecology*, **11**, 1545–1554.

Wood MJ, Cosgrove CL, Wilkin TA *et al*. (2007) Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, *Cyanistes caeruleus*. *Molecular Ecology*, **16**, 3263–3273.

Woodworth BL, Atkinson CT, LaPointe DA *et al*. (2005) Host population persistence in the face of introduced vector-borne diseases: Hawaii amakihi and avian malaria. *Proceedings of the National Academy of Sciences, USA*, **102**, 1531–1536.

Farah Ishtiaq is a research fellow at EGI, University of Oxford and is interested in host-parasite interactions, evolutionary biology and conservation biology. Laurent Guillaumot, an entomologist at Pasteur Institute, New Caledonia and is interested in the role of mosquitoes in disease transmission in South Pacific Islands. Sonya Clegg, a research fellow at Imperial College London, is interested in microevolutionary processes that underly divergence in natural systems, and uses a combination of morphological, ecological and molecular genetic approaches to examine divergence of bird populations. Albert Phillimore is a postdoc in the NERC Centre for population biology at Imperial College London and is interested in intraspecific divergence and speciation. Richard Black is in the process of a PhD focusing on the interplay between song and population divergence in birds, with a particular focus on insular white-eyes. Ian Owens, a Professor of Evolutionary Ecology at Imperial College London, studies trait evolution using wild populations and comparative approaches. Nicholas Mundy is a university lecturer in the Evolutionary Genetics group at University of Cambridge and is interested in pigmentation genetics in primates and birds, and colour vision and olfaction in primates. Ben Sheldon is a Professor at EGI, University of Oxford and is interested in evolutionary biology, ecology, behavioural ecology and host-parasite interactions.

© 2008 The Authors
Journal compilation © 2008 Blackwell Publishing Ltd