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Microsatellite and mitochondrial markers reveal strong gene flow barriers for *Anopheles farauti* in the Solomon Archipelago: implications for malaria vector control[☆]

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Abstract

Anopheles farauti is the primary malaria vector throughout the coastal regions of the Southwest Pacific. A shift in peak biting time from late to early in the night occurred following widespread indoor residue spraying of dichlorodiphenyltrichloroethane (DDT) and has persisted in some island populations despite the intervention ending decades ago. We used mitochondrial cytochrome oxidase I (COI) sequence data and 12 newly developed microsatellite markers to assess the population genetic structure of this malaria vector in the Solomon Archipelago. With geographically distinct differences in peak *A. farauti* night biting time observed in the Solomon Archipelago, we tested the hypothesis that strong barriers to gene flow exist in this region. Significant and often large fixation index (F_{ST}) values were found between different island populations for the mitochondrial and nuclear markers, suggesting highly restricted gene flow between islands. Some discordance in the location and strength of genetic breaks was observed between the mitochondrial and microsatellite markers. Since early night biting *A. farauti* individuals occur naturally in all populations, the strong gene flow barriers that we have identified in the Solomon Archipelago lend weight to the hypothesis that the shifts in peak biting time from late to early night have appeared independently in these disconnected island populations. For this reason, we suggest that insecticide impregnated bed nets and indoor residue spraying are unlikely to be effective as control tools against *A. farauti* occurring elsewhere, and if used, will probably result in peak biting time behavioural shifts similar to that observed in the Solomon Islands.

[☆]Note: Nucleotide sequence data reported in this paper are available in GenBank under accession numbers KF202340–KF202472, JN384346–JN384347, JN384354–JN384356.

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Keywords

Mosquito behaviour; Selection; *Anopheles farauti*; Population genetics; Behavioural insecticide resistance; Sex-biased dispersal

1. Introduction

The malaria vector *Anopheles farauti* sensu stricto Laveran (from here on referred to as *A. farauti*) is one of over a dozen regional cryptic species in the *Anopheles punctulatus* group found throughout the Southwest Pacific (Foley and Bryan, 1993; Beebe et al., 1994; Beebe and Saul, 1995). *Anopheles farauti* is found along the coast extending from eastern Indonesia through northern Australia, New Guinea and its associated islands, and east into the Solomon Islands and Vanuatu (Beebe and Cooper, 2002). Population genetic studies using mitochondrial and nuclear markers suggest that the centre of its diversity is in New Guinea, with populations in the Solomon Islands and Vanuatu belonging to a monophyletic lineage that is probably descended from New Guinean populations (Ambrose et al., 2012).

For the purposes of this study, the Solomon Archipelago (Fig. 1) includes the Papua New Guinea (PNG) islands of Buka and Bougainville, the Solomon and Santa Cruz Islands, and the islands of Vanuatu. The Solomon Archipelago is highly malarious with almost the entire population at risk of contracting the disease (WHO, 2012). The primary malaria vectors throughout this region are *Anopheles koliensis*, *A. punctulatus* and *A. farauti*, with only *A. farauti* present in Vanuatu (Beebe and Cooper, 2002). The use of dichlorodiphenyltrichloroethane (DDT)-based indoor residual spraying (DDT-IRS) by the Global Malaria Eradication Campaign during the 1960s and 1970s differentially impacted upon these three species. The program was highly effective in controlling *A. punctulatus* and *A. koliensis* to the extent that these species are now uncommon in the region (Taylor, 1975b; Beebe et al., 2000). However, while *A. farauti* populations were initially suppressed by DDT-IRS, they rebounded to pre-spray levels within a few years (Taylor, 1975a; Sweeney, 1983), and *A. farauti* is now the primary vector species responsible for maintaining malaria transmission in the Solomon Archipelago (Cooper and Frances, 2002; Bugoro et al., 2011a,b). Nonetheless, DDT-IRS was highly effective in reducing malaria cases in the Solomon Islands and rates of malaria infection dramatically increased when DDT-IRS was discontinued (Paik and Avery, 1973).

Differences in the biology of these three species may explain why IRS was more successful in suppressing populations of *A. punctulatus* and *A. koliensis* than *A. farauti*. Both *A. punctulatus* and *A. koliensis* are anthropophilic species that predominantly feed late at night. Thus almost all individual mosquitoes of these two species would have been exposed to the DDT used in IRS (Slooff, 1964. Observations on the effect of residual DDT house spraying on behaviour and mortality in species of the *A. punctulatus* group. Final report on a research project in West New Guinea. PhD Thesis, Institute of Tropical Medicine, University of Leyden, Netherlands; Spencer et al., 1974; Taylor, 1975b). In contrast, *A. farauti* populations traditionally exhibit more variable behaviour with biting beginning early at night and continuing throughout the night, with peak biting traditionally occurring late at night when people were asleep indoors. This “classic” *A. farauti* biting behaviour has been documented in various parts of New Guinea (Slooff, 1964; Standfast, 1967; Benet et al., 2004) and in the Solomon Archipelago–Buka Island (Cooper and Frances, 2002), San Cristobal Island (Taylor, 1975a) and the Carteret Islands (Sweeney, 1967). The behaviour and seasonal distribution of *A. farauti* at the Carteret Islands (Bougainville District, Papua New Guinea). MSc Thesis, University of Sydney, Australia).

The variation in biting time that was present in *A. farauti* populations meant that a subset of individuals – those that bite outdoors early at night – were not exposed to DDT used in IRS. Thus, the application of IRS appears to have selected for a type of behavioural resistance, resulting in a shift in the peak biting time of *A. farauti* from later to earlier in the night in New Guinea (Slooff, 1964; Spencer et al., 1974), the Solomon Islands (Taylor, 1975a; Bugoro et al., 2011a,b) and Vanuatu (Thevasagayam, 1983). Despite the removal of insecticide controls, this earlier peak biting time persists (Bugoro et al., 2011a,b), suggesting that it is heritable and that the loci governing this behaviour may be fixed in the Santa Cruz and Santa Isabel island populations and possibly in other parts of the Solomon Archipelago. Both IRS and long lasting insecticidal nets (LLIN) (the recently introduced intervention measure which also targets indoor feeding mosquitoes) select against late night indoor biting phenotypes. The resumption of a malaria elimination program in 2009 in Santa Cruz using IRS and LLIN appears to have further reinforced the early, outdoor biting activity, without any significant reduction in biting density (Bugoro et al., 2011b). In contrast, *A. farauti* populations on Buka Island were found to exhibit a “classic” late night biting behaviour – despite having undergone malaria control programs with DDT-IRS from 1961 until the early 1980s (Cooper and Frances, 2002).

The geographic variation in biting behaviour of *A. farauti* throughout the Solomon Archipelago requires further investigation, particularly in considering the efficacy of any future control measures or interventions. How can geographically structured differences in biting behaviour be sustained after the selection pressure that drove the change is removed? We hypothesize that strong directional selection combined with restricted mosquito movement and gene flow through the region permitted the independent evolution of geographically and genetically distinct populations and the subsequent maintenance of geographically structured differences in behaviour. More detailed knowledge on the population genetic structure of *A. farauti* through these islands would provide a clearer understanding of the evolution and dynamics of this behavioural insecticide resistance, facilitating better implementation and evaluation of control strategies.

2. Materials and methods

2.1. Mosquito sampling and DNA Preparation

In this study, *A. farauti* samples were collected by adult human landing catches and by dipping for larvae. Samples were collected from Manus, PNG, Buka, Bougainville, Choiseul, Santa Isabel, Ulawa, Nggela, Guadalcanal, Santa Cruz Islands and Tanna Island. The sample distribution is outlined in Fig. 1 and information on collections including biting behaviour (where known) of specimens analysed is presented in Table 1. Samples were stored frozen, in alcohol, or desiccated on silica gel. Genomic DNA from samples was extracted (Beebe et al., 1999) and identified by PCR-restriction fragment length polymorphism analysis (PCR-RFLP) of the rDNA internal transcribed (ITS2) locus to species (Beebe and Saul, 1995). Only samples identified as *A. farauti* were analysed further.

2.2. mtDNA cytochrome oxidase I (COI) sequencing and analysis

A 527 bp segment of the mtDNA COI from 139 individuals was amplified by PCR and sequenced (see Table 1 for details, GenBank accession numbers KF202340–KF202472, JN384346–JN384347, JN384354–JN384356). Sequences were aligned and edited in the program Geneious v. 5.1 (available at <http://www.geneious.com/>) and haplotype networks were constructed using TCS v. 1.21 (Clement et al., 2000) under a 95% connection limit. DNAsp v. 5 (Rozas et al., 2003) was used to estimate haplotype and nucleotide diversity for the total data and for each island sampled. Finally, we used the program Arlequin v. 3.5

(Excoffier and Lischer, 2010) to generate pair-wise fixation index (F_{ST}) values (distance method), and to test for neutrality using Tajima's D and Fu's F_s tests.

2.3. Microsatellite design, amplification and assessment

Primers for microsatellite analysis were obtained from 454 pyrosequencing of the genomic DNA of *A. farauti*. For the 454 pyrosequencing, DNA was extracted using a QIAGEN DNeasy Blood & Tissue Kit, (Qiagen, Hilden, Germany). Genomic DNA was then purified and sequenced by an external contractor, MacroGen (MacroGen, Geumchungu, Seoul). Sequences containing microsatellites were mined using MSATCOMMANDER (Faircloth, 2008) and primers were designed to amplify these microsatellite loci. Initially, 40 primers were selected to amplify dinucleotide, trinucleotide and tetranucleotide microsatellites based preferentially on two criteria: (i) number of repeats in the sequence and (ii) absence of mononucleotide repeats greater than five nucleotides in length. After testing all 40 primers on a small set of samples, the 12 best primer pairs (Table 2) were used to amplify 202 individuals (see Table 1 for sampling information).

Each locus was amplified by PCR using fluorescently labelled forward primers. The final PCR mixture contained $1 \times$ MyTaq (Bioline, UK) and 5.0–10.0 ng ($1 \mu\text{l}$) of extracted genomic DNA. The cycling involved an initial denaturation of 95°C for 3 min, then 13 cycles of 95°C for 30 s, 56°C for 40 s with a gradient decrease of $0.5^\circ\text{C}/\text{cycle}$, and 72°C for 30 s, followed by 25 cycles of 95°C for 30 s, 50°C for 40 s and 72°C for 30 s, and a final 72°C for 5 min using minimum transition times. Amplified PCR products were genotyped by an external contractor, MacroGen. Microsatellite fragment sizes were manually called with GeneMarker (Softgenetics, USA) and checked for null alleles using MICRO-CHECKER (Van Oosterhout et al., 2004).

2.4. Microsatellite analysis

2.4.1. Population genetics parameters, Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium—GenAlEx version 6.5 (Peakall and Smouse, 2012) was used to assess deviations from HWE and to estimate observed (H_o) and expected heterozygosity (H_e). The inbreeding coefficient, F_{IS} , was estimated in Genodive (Meirmans and Van Tienderen, 2004). Finally, linkage disequilibrium between loci was tested in FSTAT (available at <http://www2.unil.ch/popgen/softwares/fstat.htm>).

2.4.2. Population structure: Bayesian clustering, F_{ST} and principle coordinate analysis of genetic relatedness—The most likely number of clusters (K) was inferred using the Bayesian program STRUCTURE v. 2.2 (Pritchard et al., 2000). Simulation series were run with K ranging from 1 to 8, with 10 iterations per value of K . Each run was 1,500,000 generations in length with a burn-in of 500,000 generations. We used the admixture model with correlated allele frequencies and sample location information as priors (by site). CLUMPP (Jakobsson and Rosenberg, 2007) was then used to generate consensus bar graphs from duplicate iterations of STRUCTURE runs with the same K value. DISTRUCT (Rosenberg, 2004) was used to generate the final graphics.

Pair-wise F_{ST} values between islands were estimated in Arlequin (Excoffier and Lischer, 2010), with the significance of F_{ST} comparisons estimated by a permutation test. In addition, a principal components analysis (PCoA) was performed using GenAlEx v. 6.5 (Peakall and Smouse, 2012) in which individuals were colour coded by island, allowing visualization of genetic relatedness of individuals within and between islands.

3. Results

3.1. MtDNA analyses

Twenty-nine COI haplotypes were sampled, with an overall haplotype diversity (Hd) of 0.932. Hd per island sampled was: Buka/Bougainville, six haplotypes (Hd = 0.641); Choiseul, four haplotypes (Hd = 0.733); Ulawa, three haplotypes (Hd = 0.689); Tanna, four haplotypes (Hd = 0.442); Guadalcanal, four haplotypes (Hd = 0.625); Santa Isabel, four haplotypes (Hd = 0.900); Nggela, three haplotypes (Hd = 0.392); Santa Cruz, seven haplotypes (Hd = 0.836). Santa Cruz showed surprisingly high diversity that may be due to the samples being collected from numerous small islands. The locus did not violate assumptions of neutrality as both Tajima's D and Fu's F_s tests of neutrality were non-significant for all islands as well as for total COI data.

The mitochondrial COI haplotype network (Fig. 2) showed a clear north–south genetic division (see Figs. 1 and 2). Close genetic relationships (with shared haplotypes) occurred throughout the southern islands of Santa Isabel, Guadalcanal, Nggela and Ulawa (southern island mitochondrial group). The northern islands of Bougainville, Choiseul and Manus formed another distinct group (northern island mitochondrial group). Tanna (in southern Vanuatu) appeared to be more closely related to the northern mitochondrial group than to the southern mitochondrial group (Fig. 2) and the eastern Santa Cruz Islands were most closely related to Tanna. Shared haplotypes were not found between the northern and southern mitochondrial groups, with the genetic break occurring between Choiseul and Santa Isabel islands. The New Guinean populations were most closely related to Buka, Bougainville and Choiseul populations. Most pair-wise F_{ST} values between individual islands were relatively high and significant for the COI locus, with the exception of the Santa Isabel/Nggela comparison (Table 3).

3.2. Microsatellite population parameters, neutrality, HWE

Individuals ($n = 202$) from 10 populations were assessed at 12 microsatellite loci. No evidence of linkage between microsatellite loci was found and most loci were in HWE. While putative null alleles were found at some loci in some populations, no single locus consistently displayed null alleles (see Table 4 for a complete summary (by locus and island) of HWE, null alleles, percent missing data, number of alleles, the F_{IS} , and H_o and H_e).

3.3. Microsatellite Bayesian clustering

STRUCTURE bar plots for $K = 4$ and $K = 5$ are presented (Fig. 1) and both of these bar plots suggest that individuals from Guadalcanal and Nggela islands form a distinct group that is separate from the rest of the Solomon Archipelago. Additionally, both of the geographically distant islands (Santa Cruz and Tanna) are distinct from each other and from all other populations at both values of K . At $K = 4$, Manus, Bougainville, Buka, Choiseul, Santa Isabel and Ulawa islands form a single group, but at $K = 5$, Santa Isabel and Ulawa become separate from the other islands in this group. Thus, in contrast to the mitochondrial sequence data, the microsatellite data suggested that the strongest genetic break occurs further south, isolating Guadalcanal and Nggela from the rest of the islands of the Solomon Archipelago.

3.4. Microsatellite PCoA

In agreement with the Bayesian clustering analysis, the PCoA showed that Guadalcanal and Nggela form a distinct and coherent group separate from the rest of the Solomon Archipelago (Fig. 3). Buka, Bougainville, Choiseul, Santa Isabel and Ulawa form another

group that may contain some additional genetic stratification, as there is little or no overlap in the distribution of individuals from different islands within this group. For example, while Ulawa and Choiseul appeared genetically distinct from each other, both populations overlapped with the Bougainville population on the PCoA graph. As with the Bayesian clustering analysis, the Tanna and Santa Cruz populations were found to be clearly distinct.

3.5. Microsatellite F_{ST} based analyses

All pair-wise F_{ST} values between islands were significant and most comparisons were highly significant (Tables 3 and 5) although the STRUCTURE analyses did not suggest the presence of as many genetically distinct groups as do the F_{ST} values. Relatively low F_{ST} values suggested a close genetic association between Bougainville, Choiseul and Santa Isabel, as well as between Guadalcanal and Nggela. As expected, the highest F_{ST} values were observed in comparisons involving the distant islands of Tanna and Santa Cruz.

4. Discussion

A previous phylogeographic analysis of *A. farauti* suggested that the species' centre of diversity is in New Guinea and that a single founder event gave rise to populations in the Solomon Archipelago (Ambrose et al., 2012). This current work expands on the sampling from the previous study to focus on the population dynamics of this species in the Solomon Archipelago by including 12 additional microsatellite markers. We found evidence that *A. farauti* from the Solomon Archipelago is a single species (see mitochondrial data in Fig. 2). *Anopheles farauti* populations occupying the northern islands of the Solomon Archipelago (Manus, Buka, Bougainville and Choiseul) appear most closely related to the geographically proximal New Guinean populations. Interestingly, mitochondrial data suggests that the most southern and geographically isolated population from Tanna (Vanuatu) is more closely related to populations occurring in the northern island group than to populations of the geographically closer southern islands of Santa Isabel, Ulawa, Guadalcanal and Nggela. This genetic grouping suggests that Tanna may have been colonised by individuals from populations of the northern Solomon Archipelago. We found high haplotype diversity in Santa Cruz, which may be due to samples being collected from several small islands belonging to the Santa Cruz island group. As the Santa Cruz samples share no mtDNA haplotypes with other populations in the Solomon Archipelago, their genetic isolation is reaffirmed, with their closest mitochondrial relatives being individuals from Tanna in Vanuatu.

Both mitochondrial and microsatellite analyses detected significant population genetic structure through the Solomon Archipelago as evidenced by significant F_{ST} values between islands most likely reflecting the effects of genetic drift on small island populations. However, it appears that there may have been greater historical connectivity among some parts of the region than among others. The mitochondrial sequence data suggests a north to south genetic break separating islands north of and including Choiseul from islands south of and including Santa Isabel – there are no shared haplotypes between these regions (mitochondrial break 1; Fig. 1). This genetic break is difficult to explain given the short distance (40–50 km) separating these two islands, but may be the result of historical chance dispersal events and genetic drift.

During the last glacial maximum, between 26,500 and 19,000 years ago, the sea level is estimated to have been approximately 130 m below its current level with many islands of the region connected by land bridges (Neall and Trewick, 2008; Clark et al., 2009). At this time, this coastal breeding mosquito should have faced few physical barriers to dispersing between what are now separate islands. Despite this, we observed significant F_{ST} values between most islands at both mitochondrial and microsatellite markers. However, the

microsatellite data suggests that there is only relatively weak genetic structure between Santa Isabel and the islands to the north of it (nuclear break 2; Fig. 1). This structure could have developed since the last glacial maximum as a result of limited or no gene flow between these islands. The microsatellite data also reveals a much stronger genetic break separating Guadalcanal/Nggela (nuclear break 1; Fig. 1) from all other islands in the archipelago. This break is not supported by the mitochondrial data in which Guadalcanal/Nggela share haplotypes with both Santa Isabel to the north and Ulawa to the south. The population genetic structure through this region has probably taken thousands of years to evolve, with genetic drift on small island populations being the major driving force – it is unlikely that the different collection years (spanning 1998–2011) would significantly alter these signatures.

The observation of shared mitochondrial haplotypes between the islands Guadalcanal, Nggela, Ulawa and Santa Isabel suggests that these locations have recently been connected through the movement of females, as mtDNA is maternally inherited. However mitochondrial data suggests that Bougainville and Choiseul islands are completely separate from Guadalcanal, Nggela, Ulawa and Santa Isabel. In contrast, microsatellite analyses suggest relatively weak structure between Santa Isabel, Ulawa, Bougainville and Choiseul populations (nuclear break 2; Figs. 1 and 3), with a much stronger genetic break existing between Guadalcanal/Nggela and the rest of the Solomon Islands (nuclear break 1; Figs. 1 and 3). This discordance between the maternally inherited mtDNA and the nuclear microsatellites, which are biparentally inherited, might be explained by male-biased dispersal that could result in nuclear gene flow between islands without mitochondrial gene flow. Alternatively, this discordant genetic phenomenon may be explained by the smaller effective population size of the mtDNA (Ballard and Whitlock, 2004), which can result in faster lineage sorting of the COI locus. However, if this was the case, we would also expect to observe the populations on Guadalcanal and Nggela – that are clearly distinct based on the microsatellite data – to be fully sorted and clearly distinct in the mtDNA haplotype network, which they are not. Further resolution of this mito-nuclear discordance may require population genetics studies using Y chromosome markers to reveal genetic relationships between islands based on a marker that is exclusively paternally inherited.

A previous study of *A. farauti* in New Guinea lends additional weight to the idea that sex-biased dispersal may be responsible for the genetic pattern observed as it suggests that females may have a strong home range memory and tend to return to their natal breeding sites to oviposit, possibly making the dispersal potential of females of this species relatively low (Charlwood et al., 1988). While this was a single study, it would be evolutionarily advantageous for females to memorise features in a landscape that facilitate blood meal acquisition, resting and oviposition. This type of sex-biased behaviour, with males dispersing and females remaining within a memorized home range, could also provide evolutionary advantages to both sexes (Service, 1997), and this has been observed in other species of *Anopheles* (McCall et al., 2001). To date, little is known about the biology and behaviour of male *A. farauti*, as they are rarely encountered in the field and are difficult to collect, given that they do not blood feed and are not attracted to humans or traps. However the recent implementation of barrier screens for the co-collection of males and females may shed some much-needed light on this important issue (Burkot et al., 2013).

Populations of *A. farauti* in the Solomon Archipelago experienced strong directional selection for early evening outdoor biting as a result of DDT-IRS that was implemented during the malaria control programs in the 1960–70s. This selection resulted in the behavioural adaptation of *A. farauti* populations and impacted upon the efficacy of malaria control in the region. As mentioned previously, peak feeding times have shifted from late to early in the night on the islands of Guadalcanal, Nggela, Santa Isabel and Santa Cruz, as

well as on some islands in Vanuatu (Taylor, 1975a; Thevasagayam, 1983; Bugoro et al., 2011a,b). This behavioural shift has persisted through a number of decades after the selection pressure was removed, suggesting that the change is heritable, and that the genetic variation for time of biting has been dramatically decreased, possibly to fixation at alleles controlling this trait. Other studies on mainland PNG (Standfast, 1967; Benet et al., 2004), PNG's Buka and the Carteret Islands (Sweeney, 1967, MSc thesis, cited earlier; Cooper and Frances, 2002) have verified that the "classic" blood feeding behaviour of *A. farauti* is all night biting, with biting beginning early with peak biting occurring around midnight.

As stated above, the Buka Island populations were found to have maintained a "classic" *A. farauti* biting behaviour (Cooper and Frances, 2002), despite a DDT-IRS malaria control program of two sprays per year, that ran from 1961 until the early 1980s. As Bougainville Island is closely connected to Buka Island and is the largest island in the archipelago, it may be that it supports the largest connected population of *A. farauti* in the Solomon Archipelago, meaning that variation in the time of night biting may not have been depleted as it was in populations on smaller and more isolated islands. Additionally, due to the geographical size of Bougainville/Buka Islands, there may be subpopulations of *A. farauti* existing far away from villages that provided an inflow of genetic variability that were not depleted by the selection imposed by insecticides. Our mitochondrial and microsatellite studies detected a distinct population genetic break between the Buka/Bougainville and Choiseul Island populations (Buka has been identified as all night biting (Cooper and Frances, 2002)) and the Santa Isabel Island population (early night biting (Bugoro et al., 2011b)), see nuclear break 2 in Fig. 1), and thus the existence of gene flow barriers provides a potential explanation for how different biting phenotypes could be maintained on neighbouring islands.

Our assessment of the population structure of *A. farauti* through the Solomon Archipelago may shed light on the potential concurrent evolution of insecticide-driven behavioural adaptation on separate islands. Did this behavioural adaptation of early night peak biting behaviour occur independently in *A. farauti* populations on distinct islands? Or were the shifts in behaviour the product of rapid gene flow breaching the strong gene flow barriers apparent between islands of the Solomon Archipelago? Although gene flow of selectively advantageous alleles cannot be ruled out, we give reasons as to why the behavioral shift to early night biting in *A. farauti* may have occurred independently: (i) the genetic variation necessary for the behavioural shift was already present in populations – an early night biting trait exists naturally in all populations studied, that is *A. farauti* start biting early in the night; (ii) the shift from all night biting to early night biting could manifest rapidly – this shift was observed by Taylor in just months in the 1970s on San Cristobal Island (Taylor, 1975a); and (iii) we have identified significant genetic structure and gene flow barriers between the islands – for example, while Nggela and Guadalcanal are only separated by 40 km from the rest of the Solomon Islands, their *A. farauti* populations appear to be genetically distinct from them. The Santa Cruz Island populations are particularly remote, being isolated by more than 350 km of ocean, making gene flow at selected loci highly unlikely. While population genetics of putatively neutral loci cannot account for the evolution of adaptive behavioural changes, it may go some way towards explaining the maintenance and potential isolation of these adaptations, providing a critical insight into one of the most vexed and interesting areas of vector biology – the development of behavioural resistance to insecticide-based tools.

The World Health Organization (WHO) Global Malaria Eradication Campaign launched in 1955 was based primarily on DDT-IRS supplemented with mass drug administration (Pampana, 1969). Despite some success, including the elimination of malaria from 37 countries, malaria was not eradicated in a number of countries including the Solomon

Islands. A number of technical, administrative, financial and logistical challenges contributed to this including the development of a behavioural change in *A. farauti* (Avery, 1974; Paik and Avery, 1973, Avery, 1977). Mosquito species and populations with adequate behavioural variation adapted to DDT spraying by feeding outdoors (Reid, 1960) and towards the end of the program, transmission was being maintained in many countries by these physiologically susceptible vectors that adapted to minimize their exposure to DDT (Hamon et al., 1970; Elliott, 1972).

Today, the renewed global interest in malaria control and eradication has focused on the use of LLINs. Treated nets act in a similar manner to IRS in that they kill vectors with phenotypes that seek blood meals on humans sleeping indoors under a net. During the past decade the use of LLINs has increased across the malaria-endemic world alongside the improved treatment of infected individuals with artemisinin combination therapies (WHO, 2012). However, the problems faced during the original Global Malaria Eradication Campaign highlight the fact that the current program's success would require vector control interventions that could target species-specific vector behaviours. The Solomon Archipelago provides a good example of how selective pressure exerted by IRS can change the relative composition and abundance of mosquito species. In this case, the late night indoor biting *A. punctulatus* and *A. koliensis* populations were effectively suppressed (Taylor, 1975a; Sweeney, 1983) but *A. farauti* was not.

The widespread use of LLINs is likely to again select against late night indoor biting phenotypes. This could further drive *A. farauti* populations to feed outdoors early at night throughout their extensive Southwest Pacific distribution including PNG, eastern Indonesia, the Solomon Islands and Vanuatu, impacting on the potential efficacy of this crucial control measure. As this behavioural adaptation in *A. farauti* (and the persistence of this adaptation) to the first malaria eradication campaign suggests, vector control initiatives that only target late night biting phenotypes is unlikely to succeed in eradicating malaria where vectors such as this exist.

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References

- Avery J. A review of the malaria eradication programme in the British Solomon Islands 1970–1972. *Papua New Guinea Med.* 1974; 17:50–60.
- Avery, JG. PhD Thesis. University of Sheffield; UK: 1977. The epidemiology of disappearing malaria in the Solomon Islands.
- Ambrose L, Riginos C, Cooper RD, Leow KS, Ong W, Beebe NW. Population structure, mitochondrial polyphyly and the repeated loss of human biting ability in anopheline mosquitoes from the southwest Pacific. *Mol Ecol.* 2012; 21:4327–4343. [PubMed: 22747666]
- Ballard JW, Whitlock MC. The incomplete natural history of mitochondria. *Mol Ecol.* 2004; 13:729–744. [PubMed: 15012752]
- Beebe NW, Cooper RD. Distribution and evolution of the *Anopheles punctulatus* group (Diptera: Culicidae) in Australia and Papua New Guinea. *Int J Parasitol.* 2002; 32:563–574. [PubMed: 11943229]
- Beebe NW, Saul A. Discrimination of all members of the *Anopheles punctulatus* complex by polymerase chain reaction–restriction fragment length polymorphism analysis. *Am J Trop Med Hyg.* 1995; 53:478–481. [PubMed: 7485705]

- Beebe NW, Foley DH, Saul A, Cooper L, Bryan JH, Burkot TR. DNA probes for identifying the members of the *Anopheles punctulatus* complex in Papua New Guinea. *Am J Trop Med Hyg.* 1994; 50:229–234. [PubMed: 8116817]
- Beebe NW, Ellis JT, Cooper RD, Saul A. DNA sequence analysis of the ribosomal DNA ITS2 region for the *Anopheles punctulatus* group of mosquitoes. *Insect Mol Biol.* 1999; 8:381–390. [PubMed: 10469255]
- Beebe NW, Bakote'e B, Ellis JT, Cooper RD. Differential ecology of *Anopheles punctulatus* and three members of the *Anopheles farauti* complex of mosquitoes on Guadalcanal, Solomon Islands, identified by PCR-RFLP analysis. *Med Vet Entomol.* 2000; 14:308–312. [PubMed: 11016439]
- Benet A, Mai A, Bockarie F, Lagog M, Zimmerman P, Alpers MP, Reeder JC, Bockarie MJ. Polymerase chain reaction diagnosis and the changing pattern of vector ecology and malaria transmission dynamics in Papua New Guinea. *Am J Trop Med Hyg.* 2004; 71:277–284. [PubMed: 15381806]
- Bugoro H, Cooper R, Butafa C, Iro'ofa C, Mackenzie D, Chen CC, Russell T. Bionomics of the malaria vector *Anopheles farauti* in Temotu Province, Solomon Islands: issues for malaria elimination. *Malaria J.* 2011a; 10:133.
- Bugoro H, Iro'ofa C, Mackenzie D, Apairamo A, Hevalao W, Corcoran S, Bobogare A, Beebe N, Russell T, Chen CC, Cooper R. Changes in vector species composition and current vector biology and behaviour will favour malaria elimination in Santa Isabel Province, Solomon Islands. *Malar J.* 2011b; 10:287. [PubMed: 21961990]
- Burkot TR, Russell TL, Reimer LJ, Bugoro H, Beebe NW, Cooper RD, Sukawati S, Collins FH, Lobo NF. Barrier screens: a method to sample blood-fed and host-seeking exophilic mosquitoes. *Malar J.* 2013; 12:49. [PubMed: 23379959]
- Charlwood JD, Grave PM, Marshall TFC. Evidence for a 'memorized' home range in *Anopheles farauti* females from Papua New Guinea. *Med Vet Entomol.* 1988; 2:101–108. [PubMed: 2980166]
- Clark PU, Dyke AS, Shakun JD, Carlson AE, Clark J, Wohlfarth B, Mitrovica JX, Hostetler SW, McCabe AM. The last glacial maximum. *Science.* 2009; 325:710–714. [PubMed: 19661421]
- Clement M, Posada D, Crandall KA. TCS: a computer program to estimate gene genealogies. *Mol Ecol.* 2000; 9:1657–1659. [PubMed: 11050560]
- Cooper RD, Frances SP. Malaria vectors on Buka and Bougainville Islands, Papua New Guinea. *J Am Mosq Control Assoc.* 2002; 18:100–106. [PubMed: 12083350]
- Cooper, RD.; Taleo, G.; Yaviong, J.; Cooper, L.; Bryan, JH.; Iamaher, S. Tafea Province, Vanuatu, Tafea Entomological Survey Protocol. Australian Army Malaria Institute; Brisbane Australia: 2008. Entomological baseline data surveys on Tanna Is.
- Elliott R. The influence of vector behavior on malaria transmission. *Am J Trop Med Hyg.* 1972; 21:755–763. [PubMed: 4561523]
- Excoffier L, Lischer HEL. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour.* 2010; 10:564–567. [PubMed: 21565059]
- Faircloth BC. MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. *Mol Ecol Res.* 2008; 8:92–94.
- Foley DH, Bryan JH. Electrophoretic keys to identify members of the *Anopheles punctulatus* complex of vector mosquitoes in Papua New Guinea. *Med Vet Entomol.* 1993; 7:49–53. [PubMed: 8435488]
- Hamon J, Mouchet J, Brengues J, Chauvet G. Problems facing Anopheline vector control. Vector ecology and behaviour before, during, and after application of control measures. *Misc Publ Entomol Soc Am.* 1970; 7:28–44.
- Jakobsson M, Rosenberg NA. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics.* 2007; 23:1801–1806. [PubMed: 17485429]
- McCall PJ, Moshia FW, Njunwa KJ, Sherlock K. Evidence for memorized site-fidelity in *Anopheles arabiensis*. *Trans R Soc Trop Med Hyg.* 2001; 95:587–590. [PubMed: 11816426]

- Meirmans PG, Van Tienderen PH. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Mol Ecol Notes*. 2004; 4:792–794.
- Neall VE, Trewick SA. The age and origin of the Pacific islands: a geological overview. *Phil Trans Royal Soc London B, Biol Sci*. 2008; 363:3293–3308.
- Paik YH, Avery JG. Problem areas in the malaria eradication programme in the British Solomon Islands. *PNG Med J*. 1973; 17:61–67.
- Pampana, E. *A Textbook of Malaria Eradication*. 2. Oxford University Press; London: 1969.
- Peakall R, Smouse PE. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics*. 2012; 28:2537–2539. [PubMed: 22820204]
- Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics*. 2000; 155:945–959. [PubMed: 10835412]
- Reid JA. Secondary vectors, an obstacle to malaria eradication. *Med J Malaya*. 1960; 16:228–231. [PubMed: 13740490]
- Rosenberg NA. DISTRUCT: a program for the graphical display of population structure. *Mol Ecol Notes*. 2004; 4:137–138.
- Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*. 2003; 19:2496–2497. [PubMed: 14668244]
- Service MW. Mosquito (Diptera: Culicidae) dispersal—the long and short of it. *J Med Entomol*. 1997; 34:579–588. [PubMed: 9439109]
- Spencer T, Spencer M, Venters D. Malaria vectors in Papua New Guinea. *PNG Med J*. 1974; 17:22.
- Slooff, R. PhD Thesis. Institute of Tropical Medicine; 1964. Observations on the effect of residual DDT house spraying on behaviour and mortality in species of the *Anopheles punctulatus* group. Final report on a research project in West New Guinea.
- Standfast HA. Biting times of nine species of New Guinea Culicidae (Diptera). *J Med Entomol*. 1967; 4:192–196. [PubMed: 4383193]
- Sweeney, AW. MSc Thesis. University of Sydney; Sydney: 1967. The behaviour and seasonal distribution of *Anopheles farauti* at the Carteret Islands (Bougainville District, Papua New Guinea).
- Sweeney, AW. A review of chemical control of malaria vectors in the South West Pacific region. In: Laird, M.; Miles, JW., editors. *Integrated Mosquito Control Methodologies*. Academic Press; London: 1983. p. 143-158.
- Taylor B. Changes in feeding behavior of a malaria vector, *Anopheles-Farauti* Lav, following use of Ddt as a residual spray in houses in British Solomon-Islands Protectorate. *T Roy Ent Soc London*. 1975a; 127:277–292.
- Taylor B. Observations on malaria vectors of the *Anopheles punctulatus* complex in the British Solomon Islands Protectorate. *J Med Entomol*. 1975b; 11:677–687. [PubMed: 1123827]
- Thevasagayam, ES. *Malaria Control Strategies in the Southwest Pacific countries – reappraisal*. World Health Organization; Kuala Lumpur: 1983.
- Van Oosterhout C, Van Heuven MK, Brakefield PM. On the neutrality of molecular genetic markers: pedigree analysis of genetic variation in fragmented populations. *Mol Ecol*. 2004; 13:1025–1034. [PubMed: 15078441]
- WHO. *World Malaria Report: 2012*. World Health Organisation; Geneva: 2012.

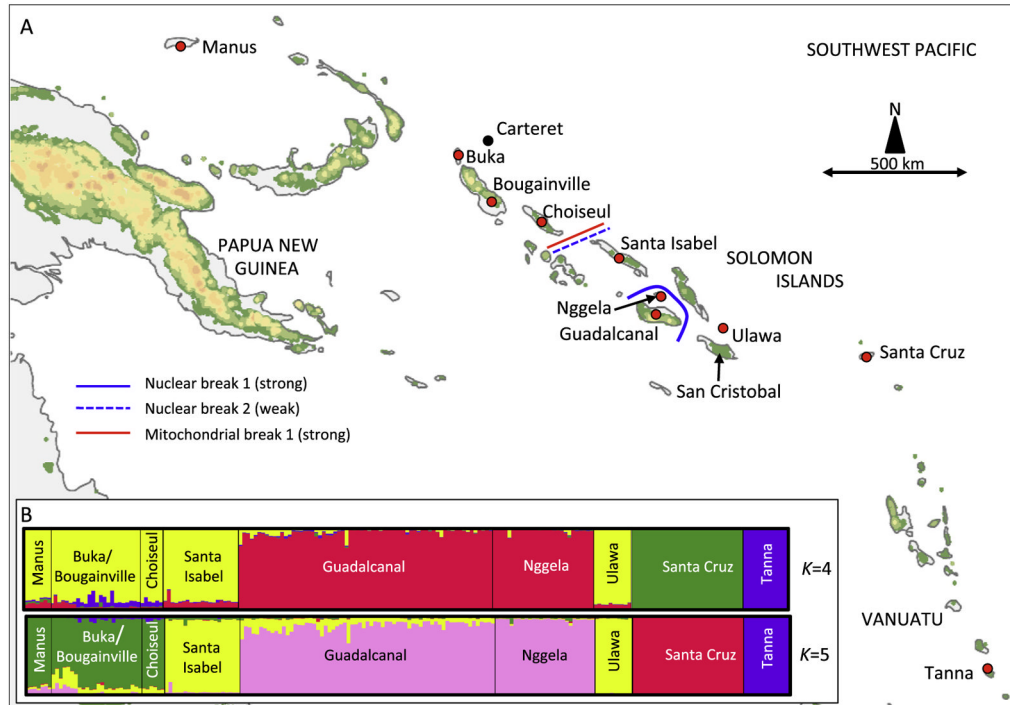


Fig. 1. Map of *Anopheles farauti* collection sites and of population genetic structure of *A. farauti* in the Solomon Archipelago. (A) Map showing the Solomon Archipelago and *A. farauti* collection sites (in red). The lines highlight the main genetic discontinuities within the Solomon Archipelago shown by microsatellites (blue line, refer to STRUCTURE plot in Figs. 1B and 3) and the maternally inherited mtDNA (red line, refer to Fig. 2). The dashed blue line indicates additional population substructure found in the microsatellite data evidenced by the Bayesian clustering method employed in STRUCTURE. (B) Bayesian STRUCTURE plots for 12 microsatellite markers run for 202 *A. farauti* individuals from 10 populations in the Solomon Archipelago. The two plots show the results from the genetic clusters value of $K = 4$ and $K = 5$. Each bar represents an individual with the colour of the bar the probability (0–1) of the individual belonging to a genetic population or cluster.

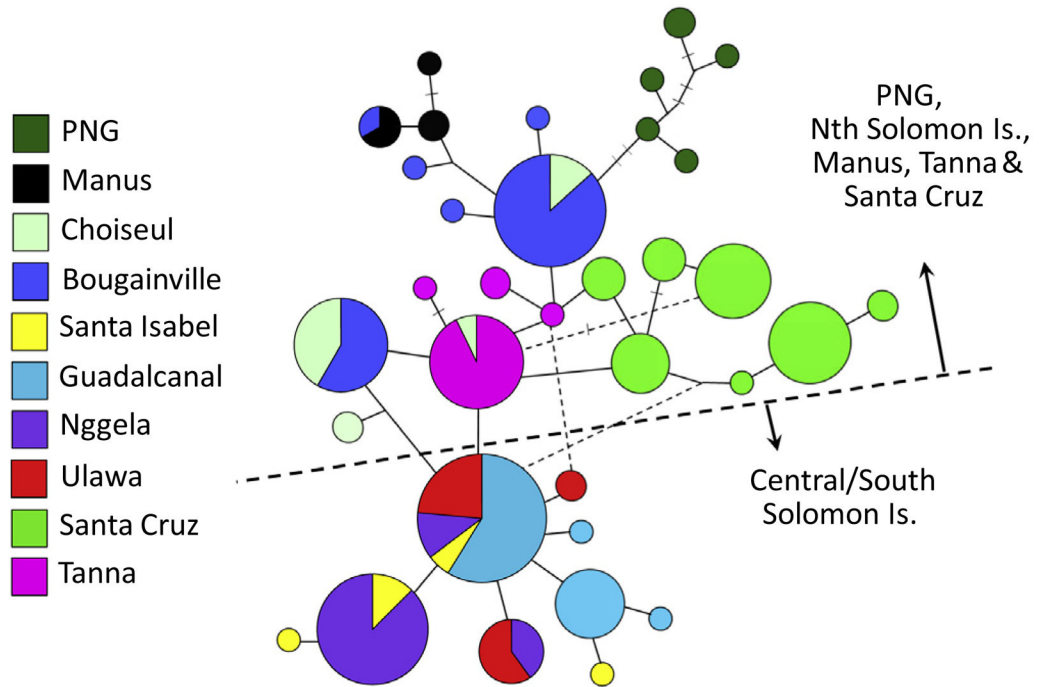


Fig. 2. mtDNA cytochrome oxidase I (COI) network for 139 *Anopheles farauti* individuals initially identified genetically to species using the rDNA internal transcribed spacer 2. Each circle represents a sequence, the size of the circle reflects the number of individuals and connections are single mutational steps between sequences with different colours representing the origin of individuals from Papua New Guinea (PNG), the Solomon Islands and Vanuatu. The network is bisected by a dotted line based on the distribution of related and shared haplotypes into a southern mitochondrial group and a northern mitochondrial group that also includes samples from Tanna (the southern limit of *A. farauti* in the region) and the Santa Cruz Islands. The upper group connects with individuals from PNG.

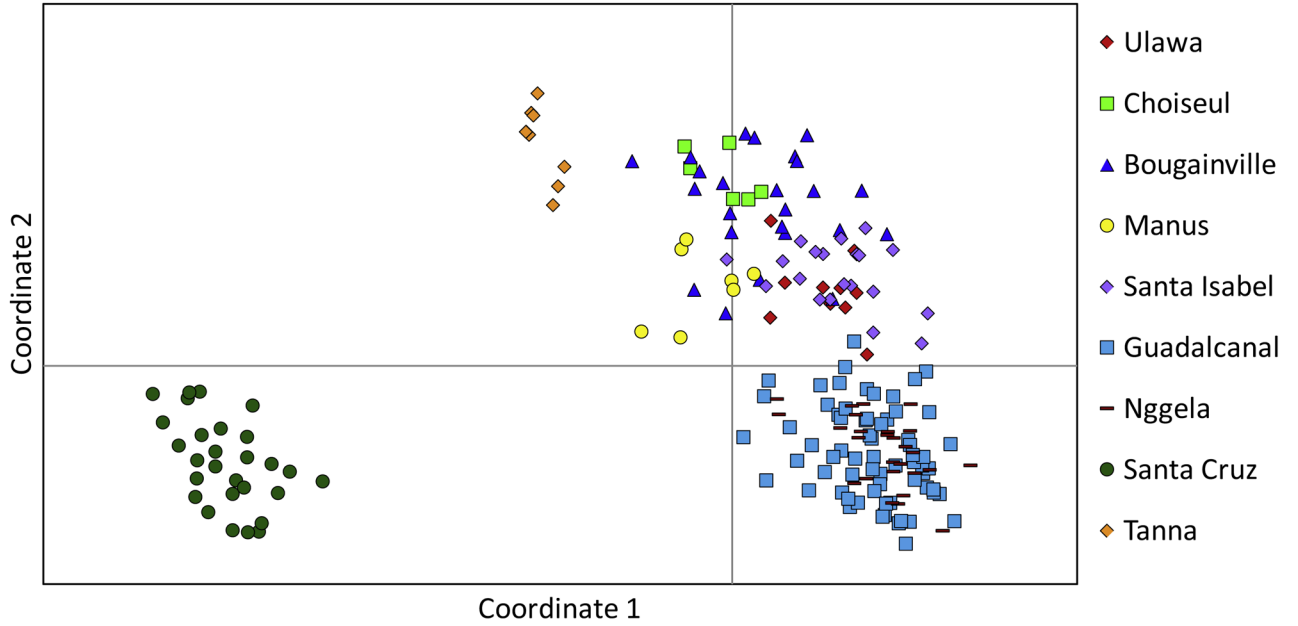


Fig. 3. Principal components analysis of 202 *Anopheles farauti* individuals assessed for 12 microsatellites. Each point represents one individual with their relative proximity to each other on the graph representing genetic relatedness. Distinct groups apparent include Guadalcanal and Nggela in the bottom right quadrant; Manus, Bougainville, Choiseul, Santa Isabel and Ulawa in the centre/top right quadrants; Tanna, top left quadrant; and Santa Cruz, bottom left quadrant. Additional substructuring is also apparent in groups within the centre/top right quadrants.

Table 1

Sampling information and the numbers of *Anopheles farauti* mosquitoes analysed for mitochondrial and nuclear genetic markers.

Map Site	Island	Year	Collection ID	Collection	Peak biting (reference)	COI	Msats
1	Manus	1998	Manus	Larval	Unknown	5	7
2	Bougainville	1999	Buka, BOU	HLC	Late Cooper and Frances (2002)	24	24
4	Choiseul	2005	Choi	HLC	Unknown	10	6
5	Santa Isabel	2010	IPP	HLC	Early Bugoro et al. (2011a)	6	20
6	Guadalcanal	1998	Gu	HLC/larval	Early Beebe et al. (2000)	17	67
7	Ulaava	2004	Ula	HLC	Unknown	10	10
8	Nggela	2011	TU	HLC	Early (Russell, unpublished data)	18	27
9	Santa Cruz	2008	SL	Larval	Early Bugoro et al. (2011b)	28	29
10	Tanna	2008	Tanna	HLC	Bimodal ^b Cooper et al. (2008)	16	12
11	PNG	1998	24f, 25f ^a	Light Trap	Unknown	6	–

COI, cytochrome oxidase; msats, microsatellites; HLC, human landing catches; PNG, Papua New Guinea.

^aCOI sequences from Ambrose et al. (2012).

^bBiting peaks 20:00–21:00 h and 02:00–03:00 h.

Table 2

Microsatellite primers developed and used in this study.

Locus	# Alleles	Range (bp)	Forward	Reverse	GenBank
DI-5	5	319–331	GTTGGTGCAGTGGGTGAAGG	TGGTCTGAAAACTGTTGCCGTG	KF202328
DI-11	15	158–196	ATGCTCTTTCGGTGTGTTGCG	GCGTTTGAAAAATCGCTGCTTC	KF202329
DI-12	5	187–195	GGTGCTAAATGTCTCAGCG	CATGGGCTGCAGTATGTTCCG	KF202330
DI-14	8	166–184	GTGAGCGCGTGATATTCCAC	ATCGGTCACTAGGGTTAGCG	KF202331
TRI-1	5	154–172	ATGTGGTCGGCTGTTTATGG	GCGTAAACACATGTGCGATC	KF202332
TRI-5	7	255–288	TTTAGCTGCAACACGACGAC	CGCGAGAAAGAGGAAACCAC	KF202333
TRI-8	6	267–303	CAGTTCATCATTCCCACGGC	GTGGATTCCGGAAGAGCAAACG	KF202334
TRI-14	9	376–403	TTCTGCCGGGATGAAATAGCG	AGCAAAGAGTCCGGTCAAG	KF202335
TRI-15	5	191–203	GGGCATGTGTTTCGGCTAGTG	GTTCCGACTCCACGGTTTAG	KF202336
TRI-19	5	214–226	AGATTAAATGTGGATTTCAGAAAG	CTTCACATAACGCCAGATCG	KF202337
TRI-24	7	124–163	TCTAGGGCGTGCAGTGTG	CAAAAGGTGGCGCAATGGG	KF202338
TRI-29	6	290–311	GCAGACGGTCTTCATTGAGC	ACGTTGGCTAGAAATTGGGAG	KF202339

Table 3

Pair-wise fixation index (F_{ST}) values between islands for the cytochrome oxidase I locus.

	Bougainville	Choiseul	Santa Isabel	Guadalcanal	Ngella	Ulawa	Tanna	Manus
Choiseul	0.256 ^a							
Santa Isabel	0.582	0.466						
Guadalcanal	0.610	0.535	0.397 ^a					
Ngella	0.688	0.652	0.032	0.614				
Ulawa	0.539	0.464	0.372	0.240 ^a	0.575			
Tanna	0.362	0.306	0.605	0.604	0.717	0.563		
Manus	0.528	0.688	0.750	0.858	0.860	0.828	0.803	
Santa Cruz	0.450	0.351	0.392	0.408	0.532	0.353	0.337	0.610

Non-significant values are in bold; all other values are highly significant ($P < 0.0001$).

^a $P < 0.01$ but > 0.0001 .

Table 4

Characteristics of *Anopheles farauti* microsatellite markers per population.

Locus	HWE, Null, % missing	[Na, Fis] (Ho, He)	Choiseul (n = 6)		Bougainville (n = 24)	
			HWE, Null, % missing	[Na, Fis] (Ho, He)	HWE, Null, % missing	[Na, Fis] (Ho, He)
Ulawu (n = 10)						
DI-5	y ^a , n, 0	[1, -] (0, 0)	y ^a , -, 0	[1, -] (0, 0)	y, n, 13	[2, -0.026] (0.1, 0.09)
DI-11	y, n, 0	[5, -0.108] (0.8, 0.69)	y, -, 0	[7, 0.216] (0.67, 0.76)	y, n, 0	[9, 0.035] (0.79, 0.8)
DI-12	y, n, 0	[3, -0.143] (0.4, 0.34)	y, -, 0	[3, 0.643] (0.17, 0.4)	y, n, 0	[4, -0.112] (0.33, 0.29)
DI-14	y, n, 0	[3, -0.333] (0.8, 0.58)	y, -, 0	[2, -0.111] (0.33, 0.28)	y, n, 17	[4, 0.055] (0.45, 0.46)
TRI-1	y, n, 10	[3, 0.632] (0.22, 0.55)	y, -, 17	[2, -0.391] (0.8, 0.54)	y, n, 0	[3, -0.070] (0.54, 0.5)
TRI-5	y, n, 0	[4, -0.009] (0.6, 0.57)	y ^a , -, 0	[1, -] (0, 0)	n, y, 4	[5, 0.474] (0.22, 0.4)
TRI-8	y, n, 0	[3, 0.268] (0.5, 0.64)	y ^a , -, 67	[1, -] (0, 0)	y, n, 0	[3, -0.015] (0.63, 0.6)
TRI-14	y, n, 0	[3, -0.216] (0.5, 0.4)	y, -, 0	[2, 0.615] (0.17, 0.38)	y, n, 13	[8, -0.068] (0.71, 0.65)
TRI-15	y, n, 0	[2, -0.059] (0.2, 0.18)	y, -, 0	[2, -0.429] (0.67, 0.44)	y, n, 8	[4, -0.120] (0.55, 0.48)
TRI-19	y ^a , n, 0	[1, -] (0, 0)	y, -, 0	[2, 0] (0.17, 0.15)	y, n, 17	[3, -0.056] (0.2, 0.19)
TRI-24	y, n, 0	[3, -0.164] (0.44, 0.36)	y, -, 0	[4, -0.111] (0.5, 0.42)	y, n, 8	[4, 0.094] (0.41, 0.44)
TRI-29	y, n, 0	[2, -0.565] (0.8, 0.5)	y, -, 17	[2, 0.273] (0.4, 0.48)	y, n, 8	[3, 0.130] (0.46, 0.51)
Santa Isabel (n = 20)						
DI-5	y ^a , n, 15	[1, -] (0, 0)	y, n, 29	[4, -0.333] (1, 0.7)	y ^a , n, 5	[1, -] (0, 0)
DI-11	y, y, 0	[6, 0.263] (0.6, 0.79)	y, n, 0	[8, 0.014] (0.86, 0.81)	y, n, 2	[11, 0.014] (0.82, 0.82)
DI-12	y, n, 5	[3, 0.237] (0.32, 0.4)	y ^a , n, 0	[1, -] (0, 0)	y, n, 2	[3, -0.151] (0.29, 0.25)
DI-14	y, n, 0	[5, 0.230] (0.55, 0.69)	y, n, 0	[3, 0.357] (0.43, 0.6)	y, n, 2	[4, -0.051] (0.55, 0.52)
TRI-1	n, n, 0	[3, -0.075] (0.6, 0.55)	y, n, 14	[4, -0.190] (0.83, 0.65)	y, n, 2	[4, 0.158] (0.55, 0.64)
TRI-5	y, n, 0	[3, 0.055] (0.5, 0.52)	y, n, 0	[3, 0.308] (0.43, 0.56)	y, n, 3	[4, -0.075] (0.52, 0.48)
TRI-8	y, n, 0	[3, -0.111] (0.75, 0.66)	y, n, 29	[2, 0] (0.2, 0.18)	y, n, 12	[5, -0.063] (0.63, 0.59)
TRI-14	y, n, 10	[4, -0.236] (0.67, 0.53)	y, n, 0	[4, -0.111] (0.71, 0.6)	y, n, 5	[8, 0] (0.66, 0.65)
TRI-15	y, n, 15	[2, -0.032] (0.12, 0.11)	y, n, 14	[2, 1] (0, 0.28)	y, n, 0	[2, -0.015] (0.05, 0.04)
TRI-19	y, n, 5	[2, 0] (0.05, 0.05)	y, n, 29	[4, 0.515] (0.4, 0.7)	y, y, 5	[4, 0.315] (0.27, 0.38)
TRI-24	y, n, 5	[4, -0.330] (0.79, 0.58)	y, n, 29	[2, 0] (0.2, 0.18)	y, n, 6	[6, -0.146] (0.49, 0.43)
TRI-29	y, n, 5	[3, -0.300] (0.68, 0.52)	y, n, 14	[2, 0.333] (0.33, 0.44)	y, n, 5	[4, -0.196] (0.61, 0.51)

Locus	Tanna (<i>n</i> = 12)		Tulagi/Volox ^b (<i>n</i> = 27)		Santa Cruz (<i>n</i> = 29)	
	HWE, Null, % missing	[<i>Na</i> , <i>Fis</i>] (<i>Ho</i> , <i>He</i>)	HWE, Null, % missing	[<i>Na</i> , <i>Fis</i>] (<i>Ho</i> , <i>He</i>)	HWE, Null, % missing	[<i>Na</i> , <i>Fis</i>] (<i>Ho</i> , <i>He</i>)
DI-5	<i>y</i> ^a , <i>n</i> , 8	[1, -] (0, 0)	<i>y</i> ^a , <i>n</i> , 0	[1, -] (0, 0)	<i>y</i> ^a , <i>n</i> , 3	[1, -] (0, 0)
DI-11	<i>y</i> ^a , <i>n</i> , 0	[1, -] (0, 0)	<i>y</i> , <i>n</i> , 0	[6, -0.078] (0.85, 0.78)	<i>y</i> , <i>n</i> , 0	[4, 0.082] (0.55, 0.59)
DI-12	<i>y</i> ^a , <i>n</i> , 8	[1, -] (0, 0)	<i>y</i> , <i>n</i> , 37	[2, -0.185] (0.35, 0.29)	<i>y</i> , <i>n</i> , 3	[2, 0.116] (0.32, 0.36)
DI-14	<i>y</i> ^a , <i>n</i> , 0	[1, -] (0, 0)	<i>y</i> , <i>n</i> , 4	[5, 0.004] (0.62, 0.61)	<i>y</i> , <i>n</i> , 3	[2, 0] (0.04, 0.04)
TRI-1	<i>y</i> ^a , <i>n</i> , 0	[1, -] (0, 0)	<i>y</i> , <i>n</i> , 0	[4, 0.126] (0.63, 0.71)	<i>y</i> , <i>n</i> , 0	[2, -0.191] (0.35, 0.29)
TRI-5	<i>y</i> ^a , <i>n</i> , 0	[1, -] (0, 0)	<i>y</i> , <i>n</i> , 0	[4, 0.177] (0.56, 0.66)	<i>y</i> , <i>n</i> , 7	[3, -0.037] (0.52, 0.49)
TRI-8	<i>y</i> ^a , <i>n</i> , 0	[1, -] (0, 0)	<i>n</i> , <i>y</i> , 26	[4, 0.444] (0.35, 0.61)	<i>y</i> ^a , <i>n</i> , 14	[1, -] (0, 0)
TRI-14	<i>y</i> , <i>n</i> , 0	[2, 0.529] (0.25, 0.5)	<i>y</i> , <i>n</i> , 7	[4, 0.051] (0.64, 0.66)	<i>n</i> , <i>y</i> , 3	[4, 0.316] (0.43, 0.61)
TRI-15	<i>n</i> , <i>y</i> , 0	[2, 1] (0, 0.38)	<i>y</i> , <i>n</i> , 15	[2, -0.048] (0.13, 0.12)	<i>y</i> , <i>n</i> , 3	[2, -0.149] (0.29, 0.25)
TRI-19	<i>y</i> ^a , <i>n</i> , 0	[1, -] (0, 0)	<i>y</i> , <i>n</i> , 7	[3, 0.040] (0.36, 0.37)	<i>n</i> , <i>y</i> , 3	[3, 0.759] (0.14, 0.57)
TRI-24	<i>y</i> ^a , <i>n</i> , 0	[1, -] (0, 0)	<i>y</i> , <i>n</i> , 0	[4, -0.111] (0.59, 0.53)	<i>y</i> , <i>n</i> , 0	[2, 0] (0.03, 0.03)
TRI-29	<i>y</i> ^a , <i>n</i> , 8	[1, -] (0, 0)	<i>y</i> , <i>n</i> , 0	[3, 0.379] (0.15, 0.23)	<i>y</i> ^a , <i>n</i> , 0	[1, -] (0, 0)

HWE, Hardy-Weinberg equilibrium; *Na*, null alleles; *Fis*, inbreeding coefficient; *Ho*, observed heterozygosity; *He*, expected heterozygosity.

^a Monomorphic allele.

^b Two separate localities on Nggela.

Table 5

Pair-wise fixation index (F_{ST}) values between islands for microsatellite data.

	Bougainville	Choiseul	Santa Isabel	Guadalcanal	Ngella	Ulawa	Tanna	Santa Cruz
Choiseul	0.048							
Isabel	0.085	0.136						
Guadalcanal	0.236	0.293	0.162					
Ngella	0.223	0.265	0.186	0.070				
Ulawa	0.148	0.238	0.069	0.205	0.246			
Tanna	0.360	0.533	0.419	0.472	0.495	0.578		
Santa Cruz	0.486	0.610	0.502	0.445	0.462	0.575	0.713	
Manus	0.140	0.172	0.201	0.261	0.245	0.264	0.539	0.515

Non-significant values are in bold; all other values are highly significant ($P < 0.001$).