epidemic diseases to Aneityum reported consistent use of nets throughout the year. Several informants stated that they were able to control breeding sites or prevent mosquito bites. But they also reported that they were not able to control the movement of people.

> *"We can avoid mosquito bites, but we cannot control people. So, everyone on this island should be tested more frequently to maintain a healthy environment, because many strangers walk around the village." (Male KII, Analgaut)*

A few elder key informants noted that knowledge of health benefits gained through their experience encouraged participants to sustain the miracles that they saw in the 1990s. A few elderly interviewees and some young discussants did not express enthusiasm for ME activities. They insisted that nobody died of malaria for a long time on the island. Tangible benefits from tourism did not inspire participants to engage in ME activities. Some typical comments were:

> *"We do not think a malaria-free environment will increase the number of tourists. We do not see the relationship between a malaria-free island and tourism. Why do you ask?" (Female FGD, Analgaut)*
> *"We prevent malaria for ourselves. We do not prevent malaria for foreigners." (Male KII, Analgaut)*

Theme 2: Skills

Skills including those needed for behavioral maintenance, problem-solving and leadership were widely reported by participants.

Skills necessary to maintain the behavior change Most participants, regardless of age or gender, reported being motivated to prevent malaria for their health and development. Several key informants noted that people on the island maintained a pattern of behavior. A few female informants stated that the access to and use of intervention services helped sustain changes since the 1990s. Many participants, regardless of age or gender, reported sleeping under a net even during the dry season when mosquito breeding sites are not abundant. Village and household cleanliness were frequently reported as essential for ME. All participants reported using Western medicine to treat malaria. All participants noted that they recognized the beneficial effect of Western medicine through the interventions and they preferred to use it rather than *kastom* medicine (traditional or herbal medicine). Some participants stated that they used *kastom* medicine only for various symptoms. Rapid diagnostic tests (RDTs) were not utilized by participants. All participants noted that they preferred malaria diagnosis using microscopy for a high fever. A few participants reported delays due to weather and community infrastructure.

> *"Three to five times a week, people turned up late for a test because of bad weather and location." (Male KII, Analgaut)*
> *"When it rains hard, it is difficult to go to the main village. I will check my blood later." (Male IDI, Port Patrick)*

Problem-solving skills A group of young female participants mentioned financial burdens (i.e., education and living costs) as a barrier to purchasing new nets and accessing health services. In order to solve these problems, some of the young female participants reported that they would seek help from their extended family.

> *"Many people travel on foot. If people have money, they will take a taxi boat from Port Patrick to the dispensary or they will take an airplane to the hospitals on Tanna and in Port Vila. They can borrow money from their extended families. Otherwise, they will walk or ask strong men to help." (Female KII, Analgaut)*
> *"Travelling by taxi boat is expensive. All my families walk to the dispensary. We have relatives living in the main village, so we can stay at their house." (Female IDI, Port Patrick)*

Because some young people on the island did not use or buy nets, many older participants emphasized the importance of information sharing for ME.

> *"Some young people do not buy nets. They are crazy. Information sharing is very important." (Female IDI, a small village near Analgaut).*

Written health education materials were occasionally reported as barriers to information sharing. Most participants reported preferring face-to-face communication.

> *"Some people do not read the notice. Picture-based instructions will help promote a better understanding. Several languages are spoken on this island. That sometimes makes things difficult. Face-to-face communication is the best way to communicate with people." (Female KII, Analgaut)*

Several informants noted the importance of education to support people who did not understand or respond to health messages. A few elderly female key informants stated that they were good performers to raise awareness.

Leadership skills A few elderly male key informants and interviewee explained that a decline in the population on the island due to 'blackbirding (the recruitment of South Pacific islanders to secure cheap labour)' in the 1800s and imported diseases undermined their *kastomary* system. An elderly male interviewee stated that chiefs tried to strengthen *kastomary* system and practices through education and awareness. Some elder key informants reported

that they were eager to educate youth so that they can assume future leadership roles in the communities.

"We will prevent malaria. In the past, many people were killed or taken. Now we try to strengthen our system and develop our future leaders." (Male KII, Analgaut)

Many informants stated that they were willing to promote community health. Some of them reported that they needed more information to be independent.

"To keep people informed is very important, then the communities can do for themselves." (Female KII, Analgaut)

A few informants and interviewees noted that they needed training for the communities.

"I will go to school to be a nurse, because we have no female nurse on the island." (Female IDI, Analgaut)
"Most people need money on this island, and I have five children. I work on a contract basis. I want to contribute to the communities. I need more training to become a mentor. And then I will train younger generation." (Male KII, Analgaut)

Participant perceptions related to social-contextual resources

Social-contextual resources consisting of 'social services (organizational structure)' and 'support networks (social structure)' promoted ME efforts.

Social services

A local health coalition provided ME services.

Theme 3: An existing local health coalition

Within the local organizational structures of the islands (i.e., the health committee that included local authorities), a health coalition was established to provide ME services to community members (Fig. 1). These services included health promotion, awareness, education, treatment, vector control measures (such as supplementary ITN distribution, the draining of swamps and the use of larvivorous fish in ponds) and community-based surveillance. Community-based surveillance was conducted by a malaria microscopist along with the provincial malaria team. The health committee recently added a male trainee (a microscopist) to ensure availability of staff for robust surveillance. Still, several participants addressed the challenges of screening due to lack of equipment. Understaffed and undersupplied facilities were the most commonly cited barriers to providing and accessing ME services among participants. The supply ordering systems between Aneityum and Tanna Islands reportedly did not always work. Some key informants noted the importance of prevention.

"Health Facilities have no drugs, no water and no female nurse. A community radio is broken. We have many problems so prevention is very important. Prevention is better than cure." (Female KII, Analgaut)

A retired female nurse occasionally assisted sick people. Some female participants stated that they preferred to share their health problems with female health staff. They reported that a retired female nurse was only available on the island. This finding identifies the importance of same gender health staff to establish communication channels and promote engagement.

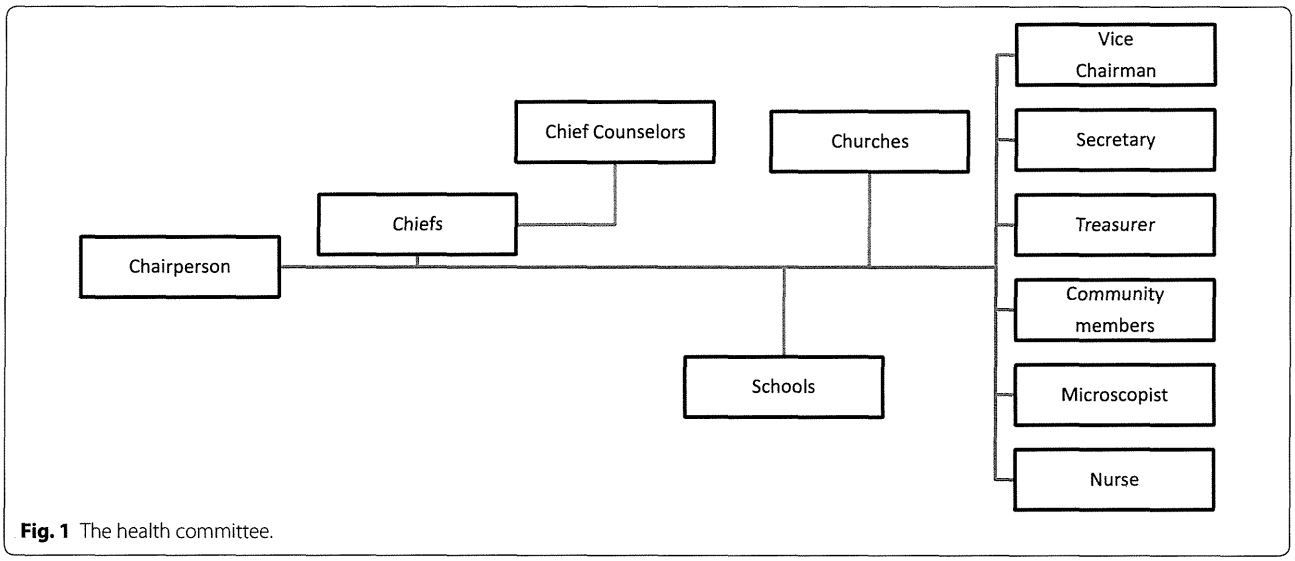

Fig. 1 The health committee.

"I talk with my husband when I have health problems. And my husband will talk to a male nurse. I do not want to talk about my health with other males." (Female IDI, Port Patrick)

Most key informants held multiple leadership positions in their communities and some informants were closely related to one another, which reportedly strengthened a local health coalition. Faith-based activities and school-based activities promoted ME activities. Awareness campaigns were conducted by health staff, church leaders and teachers. The tidy village campaigns were mainly organized by schools to ensure cleanliness.

"We just decided to start a tidy village campaign this year. This campaign is held on Tuesdays. Households, students and parents have a clean-up day. The competition takes place every two to three months. Primary and secondary students will pick up trash and count the number. Area is divided into four parts. We manage our campaign." (Female KII, Analgaut)

Many participants stated that chiefs promoted community health.

"When a chief blows a conch shell, people gather. He gives information to all members once in a month. Now, this village has developed. Many health staff visit this village. Chiefs work together with them." (Female IDI, a small village near Analgaut)

Some male participants noted that local system and norms played a role in decision making process.

"My clan, the biggest clan on the island, has more than 300 members. We talk and decide everything." (Male IDI, Analgaut)
"There are three or four tribes in my area. We share the land and live close. So we help each other to solve the health problems." (Male IDI, Port Patrick)
"People follow their family rules and practice their religion to live a healthy lifestyle. Head of the family is not a job for a woman. Women are responsible for child-rearing, cooking and weaving. Men make the final decisions." (Male IDI, Unmet)

Support networks

Members of networks were sources of information and assistance for ME efforts.

Theme 4: Sources of information

All participants belonged to multiple support networks such as chiefdoms, lineages, kin groups, health, religion, education or sports. It was commonly stated that participants were likely to have frequent face-to-face contact with network members, and they preferred face-to-face advice.

Figure 2 shows that participants share information on malaria through both formal networks (e.g., public health services, chiefs, churches and schools) and informal networks (e.g., kin and friends). These networks were reportedly used for delivery, exchange and gathering. Communication channels for ME included public notice, posters, leaflets, community meeting, the *Nakamal* (a traditional meeting place or kava bar), church services, drama, events, storytelling, workshops, school curriculum, face-to-face conversations, mobile calls and text messages. Some participants owned mobile phones or personal radios. Community meetings were commonly thought to be the best way to mobilize all community members and share malaria information.

Theme 5: Sources of assistance

All participants reported that help was available to them from somewhere in their networks. It was commonly stated that kin in the neighborhood mutually supported one another.

"For example, a big sister will ask me to help if her daughters are unavailable. I will ask her to help me in time of need. Some people will ask their cousins or relatives. We are neighbors and friends. We support each other when someone has a health problem. This is our system." (Female KII, Analgaut)

All IDIs reported they had three or more kin (cousins of similar age and gender, siblings, parents and grandparents) in their neighborhood (estimated to be within a five-minute walk) to turn to for help. Two female interviewees named each other as friends (kin) to count on. Supportive relationships reportedly maintained a healthy lifestyle among members.

"When I have health problems, more than ten friends in my neighbourhood support me. Friends mean kin or extended families to support one another. If I shout for help, they will come and take me to the dispensary." (Male IDI, Port Patrick)

Discussion

Community resources (personal and social-contextual resources) on Aneityum were identified, developed and poured into eliminating malaria by the local people in the process of community engagement. Interrelated individual and structural forms of capacity facilitated the effective mobilization and utilization of resources for their health and well-being. These multilevel empowerment responses across individual, organizational and community structural levels in community engagement

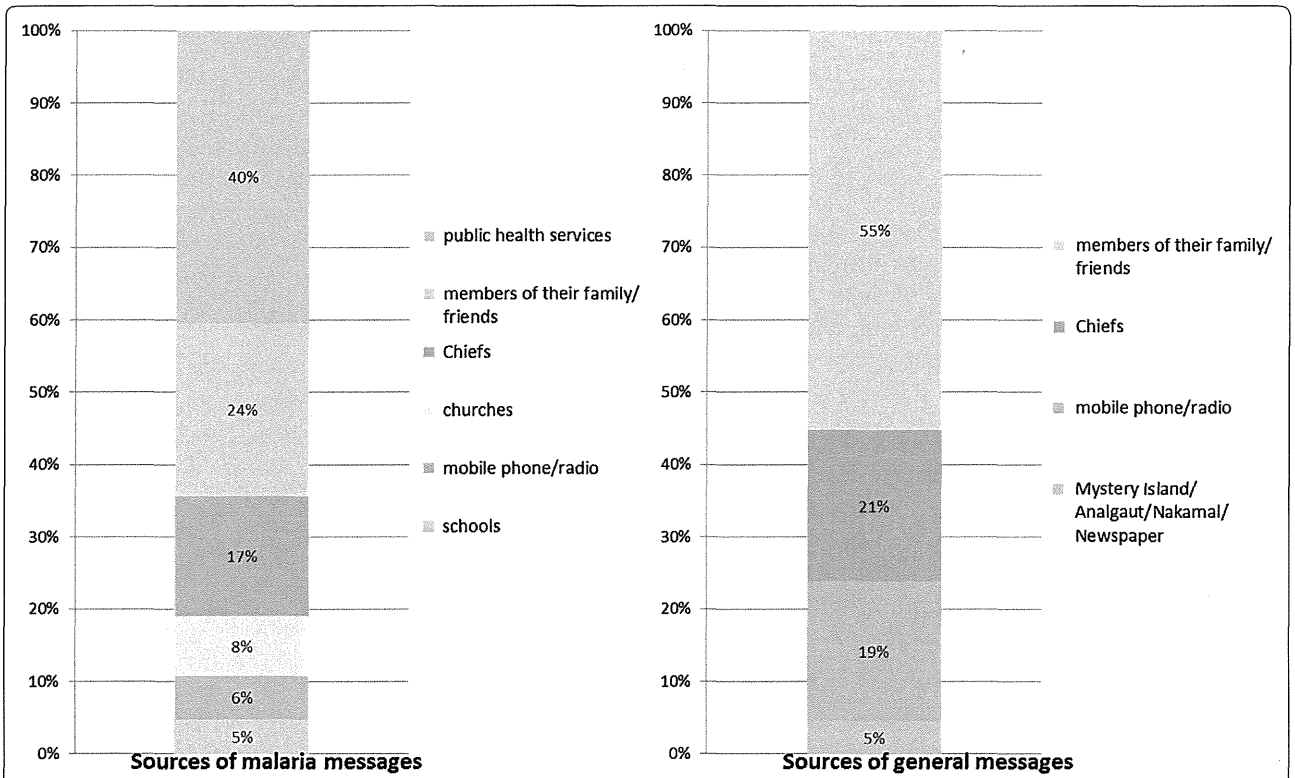

Fig. 2 Sources of information.

continuum are explained further below in the context of HET.

The recognition and development of personal resources

The results of this study showed that people who acknowledged the threat (severity and susceptibility) were intrinsically motivated and committed to engaging in active efforts in order to reduce importation and outbreak risk by developing individual level ME knowledge and skills, which might enhance their self-efficacy to further promote and maintain health-enhancing behaviors. People used their problem solving techniques to access, share or develop ME resources. People who experienced past malaria endemicity tended to focus their attention on sustaining engagement in ME and empowering the next generation. People who utilized the resources available for ME gained control over their behavior and their lives. These findings do not fully support previous findings, which have shown that the communities of Aneityum are motivated both by the benefits to the health of the islanders and by the fact that cruise ships call at Mystery Island and would stop doing so if malaria returned [13]. Although the sale of tourism products, such as seafood, handicrafts and docking fees were widely recognized as sources of income or sources of financial assistance for community-based activities (e.g., school fees) in this study, there is clear indication that the health of the islanders is more important than extrinsic (material) incentives.

The provision and utilization of social services (social-contextual resources)

Communities where vector-borne diseases are endemic often lack adequate institutional systems and structures to facilitate community engagement in disease control strategies [4]. However, existing organizational and leadership structures on Aneityum facilitated community engagement in ME. A health coalition on Aneityum provided ME services, promoted collaboration between health staff and community leaders (such as chiefs, church leaders, teachers, youth leaders and group leaders) and coordinated ME activities. It generates resources for ME and facilitates the communication flow through its overlapping and interrelated networks, which increases resource mobilization and utilization on the island. In Isabel Province, Solomon Islands, a local leadership structure known as 'the Tripod', a cooperative alliance between village Chiefs, the provincial government and the Church of Melanesia (Anglican) along with the Mothers' Union (an international charitable network of

Anglican women), has a strong influence on communities with the spirit of togetherness in community tasks and activities for ME [3, 20]. These remote Western Pacific island communities on Aneityum Island and in the Isabel Province demonstrate that existing local organizational structures built around their norms, customs, beliefs and values foster community self-reliance and ownership of health initiatives to implement the community engagement strategies in the community-based interventions (i.e., outreach and health promotion activities and service delivery) in a locally appropriate, feasible and sustainable way.

The recognition and utilization of support networks (social-contextual resources)

Existing networks facilitated service delivery, service utilization and resource mobilization. The more membership networks people recognized and belonged to, the more resources they could access or share. Networks of mutually supportive relationships (aligned with social capital concepts in which networks and the associated norms of reciprocity have value [21]) fostered social cohesion and solidarity in traditional communities on Aneityum through collaboration. Communities that are rich in social capital (e.g., those in the Isabel Province and on Aneityum Island) are more likely to promote health-enhancing behaviors [21, 22]. This study indicates that the existing local social capital on Aneityum continues to foster a strong sense of community among members, which contributes to solving community-identified problems to develop preventive health behaviors and create health-enhancing environments for individual and collective well-being.

Community engagement as a 'means' and as an 'end'

In analyzing changes in community engagement in ME since the 1990s, it is clear that 'community engagement as a means' to achieve predetermined objectives within a specified time-period facilitates 'community engagement as an end' where the communities sustain the capacity development process to exercise more control over their own health and environment. The means approach is usually adopted in disease control programs [4, 23, 24]. However, this study indicates that both approaches (the means and ends approaches) can coexist [24] and reinforce each other in malaria elimination program on Aneityum to sustain the changes.

Prescription for sustaining community engagement

This study demonstrates how over time the communities have taken steps to internalize key issues and act in their own self-interest in a goal-oriented intervention (i.e., malaria elimination) for their health and well-being in the community engagement continuum. These local capacity development efforts facilitate empowerment. Community engagement produces empowerment and at the same time, it is only possible with empowerment [4]. Community engagement needs to mature into empowerment to sustain interventions [8]. Empowerment is both an outcome and a process of community engagement [9]. This process activates momentum for change. Momentum grounded in and inspired by the local context will continue to help achieve targets even after withdrawal of the external agency and promote endogenous development in a lasting, sustainable way.

To replicate the success of Aneityum on larger islands with more complex socio-cultural and environmental contexts (such as Tanna Island, Vanuatu), Atkinson et al. wrote a prescription for policy makers (e.g., government officials, health professionals and researchers) to build and maintain community engagement as a means to eliminate malaria [1]. This includes mobilizing local resources (leaders and networks), strategies to overcome gender barriers, provision of outreach to remote communities, monitoring and knowledge acquisition support through media campaigns to advice communities to eliminate malaria or address barriers to engagement [1]. Community engagement organized for the purpose of taking prescribed actions in the paternalistic approach of imposing interventions on communities through participatory methods is widely used, but actions are not always sustained due to mismatch between top-down program requirements and local contexts (custom, environment, motivation, needs and priorities) [2, 4, 6, 24]. People cannot be fully empowered by the health promoters or government officials; they can only empower themselves to leverage their own resources and make appropriate plans and decisions in the local context through a process of engagement [4, 9, 25]. The enhanced empowerment in the active community engagement continuum may facilitate a smooth transition from externally driven interventions to community-led interventions.

Limitations

This prescription will most likely be written in flourishing traditional and religious communities that may be economically impoverished yet rich in community-based social capital through community leadership structure and densely knit support networks. Community engagement can take many forms and will work for interests in different ways in various situations. The qualitative results are limited to be generalized to the wider population of Aneityum. However, a range of perceptions and attitudes are captured to explain a health empowered community response in this study. Participant cancellation and time constraints faced by researchers have

negative impacts on the validity of the results. The definitions of personal and social-contextual resources proposed by HET are used in this study. This study does not fully take into account economic issues including those associated with tourism that may also influence community engagement in ME. The impacts of globalization on social capital (negative and positive effects) should be further investigated on Aneityum. Responses and priorities may be influenced by the presence of the research team and local leaders. Some responses relating to elimination efforts may be subject to social desirability bias. There will be some degree of loss of nuances and depth because of the simultaneous and direct translation (from Bislama to English) and non-native interactions between a female researcher, local facilitators and participants, which may hinder free expression and accurate representation of views.

Conclusions

Community engagement, which facilitates local personal and social-contextual resource development, has potential for target achievements and multilevel empowerment through community-based capacity development processes. Self-empowered communities have written and will continue to write a 'prescription' for sustaining high levels of engagement.

Additional file

Additional file 1. Questions for interviews.

Authors' contributions

NW designed the survey. AK, JKL, GT, TT, PSL and SY coordinated the field work. Ethical clearance was obtained by JKL, AK, GT, SY and NW. NW and SY conducted qualitative research. Qualitative data analysis was carried out by NW, SY and AK. Manuscript drafting was carried out by NW with contribution from NBCS and AK. All authors read and approved the final manuscript.

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Compliance with ethical guidelines

Competing interests

The authors declare they have no competing interests.

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Novel Mutations in K13 Propeller Gene of Artemisinin-Resistant *Plasmodium falciparum*

Rie Isozumi, Haruki Uemura, Isao Kimata, Yoshio Ichinose, John Logedi, Ahmeddin H. Omar, Akira Kaneko

We looked for mutations in the *Plasmodium falciparum* K13 propeller gene of an artemisinin-resistant parasite on islands in Lake Victoria, Kenya, where transmission in 2012–2013 was high. The 4 new types of nonsynonymous, and 5 of synonymous, mutations we detected among 539 samples analyzed provide clues to understanding artemisinin-resistant parasites.

The worldwide spread of malaria parasites with resistance to antimalarial drugs has been a serious concern over the past few decades. During the 2000s, *Plasmodium falciparum* parasites acquired resistance to key drugs, such as chloroquine and sulfadoxine–pyrimethamine, in many malaria-endemic countries, including Kenya (*1*). Artemisinin-based combination therapy (ACT) has been introduced in most malaria-endemic countries and is the first-line therapy. However, the first clinical cases of artemisinin resistance in western Cambodia were reported in 2008 (*2*), and *P. falciparum* with reduced in vivo susceptibility to artesunate in western Cambodia was reported in 2009 (*3*,*4*). On the basis of these findings, genome-wide analyses of artemisinin-resistant P. falciparum isolates found strong correlations between a mutant allele in the K13 propeller, in vitro parasite survival rates, and in vivo parasite clearance rates; these correlations indicate that mutations in the K13 propeller (especially C580Y, R539T, and Y439H) are important determinants of artemisinin resistance (*5*,*6*). Analysis of parasites from several Cambodian provinces indicated that K13 propeller mutations are rarely observed in samples from provinces without documented resistance but are prevalent in provinces with reported resistance (*6*,*7*).

In Kenya, ACT was introduced as first-line therapy for *P. falciparum* malaria in 2004 (*1*). However, various types of antimalarial drugs—including chroloquine, sulfadoxine–pyrimethamine, and artemether/lumefantrine—are available for purchase at pharmacies without physicians' prescriptions. In this study, we describe some K13 propeller mutations of *P. falciparum* parasites from western Kenya.

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The Study

The research was conducted at 4 islands on Lake Victoria (Kibuogi, Ngodhe, Takawiri, Mfangano) and 1 shoreline community of Mbita District (Ungoye) in western Kenya (Figure). In this area, the $PfPR_{2-10}$ (community *P. falciparum* parasite rate standardized to the 2- to 10-year age group) was reported in 2009 to be >40% (*8*). Although in some area of Kenya malaria has decreased, its prevalence remains high in the Lake Victoria basin because of the lake environment (*8*–*10*). In 2009, a total of 50%–70% of households owned insecticide-treated bed nets (*11*), which substantially reduce the risk for transmission of malaria parasites by providing barriers against mosquitoes. Although malaria is annually more prevalent in the 2 wet seasons (March–June and October–November) (*9*), in the study sites, it is highly endemic throughout the year.

Filter paper blood spots were collected from participants (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/21/3/14-0898-Techapp1.pdf) during population-wide cross-sectional malaria surveys conducted in February and August 2012 and August 2013 at the 5 study sites. We obtained ethics approval from the Kenyatta National Hospital and the University of Nairobi. All study participants provided informed consent.

Parasitic DNA was extracted from the filter paper (*12*), and *P. falciparum* DNA was detected by a mitochondrial DNA-based PCR (*13*). Sequencing of the K13 propeller gene was attempted on the diagnostic PCR-positive specimens (online Technical Appendix). The prevalence of *P. falciparum*, as determined by PCR, in the rainy and dry seasons was 7.2%–26.2% and 6.5%–15.5% on the 3 small islands (Kibuogi, Ngodhe, and Takawiri), 47.3% and 31.4% on Mfangano Island, and 38.4% and 41.9%–64.0% in Ungoye, respectively (Table 1).

We successfully analyzed 539 samples for the K13 propeller gene. Nine new types of point mutations were identified among these samples (Table 2). Participants infected with parasites harboring a mutation on the K13 propeller gene are listed in online Technical Appendix Table 2. The sequences reported in this study have been deposited in the DDBJ/EMBL/GenBank databases (accession nos. AB936059–AB936067).

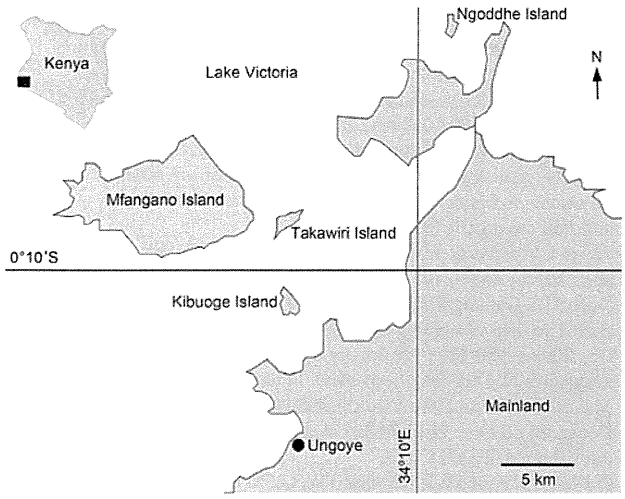

Figure. Study sites for investigation of K13 propeller gene in *Plasmodium falciparum*, Mbita District, Kenya, 2012–2013. Inset shows location of study area in Kenya.

Table 1. Prevalence of *Plasmodium falciparum* and analysis of K13 propeller gene, Mbita District, Kenya, 2012–2013

Time	Study site	Total no. samples	PCR positive, no (%)*	K13 propeller gene analyzed, no.
2012 Feb	Kibuogi	130	34 (26.2)	21
	Ngodhe	250	18 (7.2)	5
	Takawiri	250	34 (13.6)	19
	Mfangano	427	202 (47.3)	138
	Ungoye	250	96 (38.4)	69
2012 Aug	Kibuogi	195	17 (8.7)	10
	Ngodhe	232	36 (15.5)	18
	Takawiri	230	15 (6.5)	9
	Mfangano	706	222 (31.4)	145
	Ungoye	248	104 (41.9)	65
2013 Aug	Ungoye	250	160 (64)	40

*PCR detected *Plasmodium falciparum* only.

Conclusions

We detected 4 novel nonsynonymous and 5 novel synonymous mutations in the highly conserved K13 propeller gene of *P. falciparum* parasites from western Kenya. Ariey et al. noted that the frequency of mutant alleles strongly correlated with the prevalence of day 3 positivity after ACT treatment in humans in Cambodia and that those mutations reflected positive selection (*6*). That study found 17 mutant alleles in the K13 propeller gene. Among them, C580Y, R539T, and Y493H were prevalent and strongly related to in vivo delayed parasite clearance. In our study, all the mutations found differed from those reported in Cambodia, and mutant alleles were not always observed in the proceeding seasons, so some mutations could be occasionally introduced. Most of those mutations are not suitable for the life cycle of parasites, and only a few suitable for survival under the conditions of artemisinin selection pressure could be selected.

We observed no identical mutations at >2 of these 5 study sites. Furthermore, only 1 type of mutation, A578S from Mfangano Island, was detected during 2 seasons, whereas other mutations were not observed in the next season, half a year later. Any family relations were not identified among the 4 participants harboring A578S mutation in February 2012 at Mfangano Island. Point mutations can occasionally occur on the *P. falciparum* K13 propeller gene as a standing variation, but most of the isolates that recently acquired the mutation may disappear because of some fitness disadvantage or the effect of a random genetic drift (*14*).

Malaria parasites grow and multiply at 2 different biologic stages in humans and mosquitoes. Therefore, isolates with new mutations must adapt to both circumstances. We detected the mutant allele A578S in the K13 propeller gene in 2 consecutive seasons on Mfangano Island. This mutation, which modifies amino acids from being hydrophobic to hydrophilic, is close to the prevalent single nucleotide polymorphism C580Y from Cambodia that is thought to be necessary in protein–protein interactions, which could affect artemisinin susceptibility. The genotype analyses of the parasites from this island are critical to understanding the role of this mutation and ACT efficiency in this geographic area.

Our K13 propeller sequence analysis of *P. falciparum* parasites from a malaria-endemic area in Kenya did not detect the predicted artemisinin-resistant genotypes, but we observed some temporal substitutions. A limitation of our study was that the sample size was insufficient to specifically provide an understanding of this result. The accumulation of data from this region and from other

Table 2. Observed mutations in the K13 propeller gene in *Plasmodium falciparum*, Mbita District, Kenya, 2012–2013

Mutation	Amino acid change and location	Genetic change	Study site (no. isolates)		
			2012 Feb	2012 Aug	2013 Aug
Nonsynonymous	M442V	ATG →GTG		Mfangano (1)	
	N554S	AAT →AGT	Ungoye (1)		
	A569S	GCA →TCA			Ungoye (1)
	A578S	GCT →TCT	Mfangano (4)	Mfangano (1)	
Synonymous	C439C	TGC→TGT	Ungoye (2)		
	S477S	TCT →TCG	Takawiri (1)		
	Y500Y	TAT→TAC		Mfangano (1)	
	N531N	AAT →AAC			Ungoye (1)
	G538G	GGT→GGA	Mfangano (3)		

malaria-endemic areas will increase our understanding of the relationship between the K13 propeller gene and artemisinin resistance. Monitoring these molecular markers and the efficacy of antimalarial drugs is critical for increasing understanding of artemisinin resistance and predicting its spread. This study identified clues that are essential in understanding artemisinin-resistant parasites.

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RESEARCH ARTICLE

Plasmodium vivax and *Plasmodium falciparum* at the Crossroads of Exchange among Islands in Vanuatu: Implications for Malaria Elimination Strategies

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Abstract

Understanding the transmission and movement of *Plasmodium* parasites is crucial for malaria elimination and prevention of resurgence. Located at the limit of malaria transmission in the Pacific, Vanuatu is an ideal candidate for elimination programs due to low endemicity and the isolated nature of its island setting. We analyzed the variation in the merozoite surface protein 1 (*msp1*) and the circumsporozoite protein (*csp*) of *P. falciparum* and *P. vivax* populations to examine the patterns of gene flow and population structures among seven sites on five islands in Vanuatu. Genetic diversity was in general higher in *P. vivax* than *P. falciparum* from the same site. In *P. vivax*, high genetic diversity was likely maintained by greater extent of gene flow among sites and among islands. Consistent with the different patterns of gene flow, the proportion of genetic variance found among islands was substantially higher in *P. falciparum* (28.81–31.23%) than in *P. vivax* (-0.53–3.99%). Our data suggest that the current island-by-island malaria elimination strategy in Vanuatu, while adequate for *P. falciparum* elimination, might need to be complemented with more centrally integrated measures to control *P. vivax* movement across islands.

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Introduction

Renewed commitment to control malaria over the last decade has resulted in major reductions in case incidence and disease mortality rates, and 32 of 99 countries with endemic malaria are pursuing an elimination strategy [1,2]. Outside of sub-Saharan Africa, *Plasmodium vivax* infections present unique and additional challenges for elimination due to the parasite's propensity to relapse and the limitations of primaquine [1,3]. Further, malaria resurgence has the potential to undermine control and elimination efforts [4–6]. To this end, parasite population genetics studies are fundamental in identifying routes of transmission and gene flow, such that appropriate strategy for control and intervention might be implemented [7].

Islands provide an ideal model for natural ecological experiments and present a great opportunity for intervention studies. Vanuatu is an archipelago consisting of 68 inhabited islands located at the southeastern limit of malaria transmission in the Pacific. Malaria is mainly hypo- to meso-endemic, with a general decrease in annual parasite incidence (API) from the northwest to the southeast. *P. falciparum* and *P. vivax* are the predominant species, with a slightly higher prevalence of the latter especially on the southern islands [8]. Since the early 1990s, transmission rates have decreased as a result of malaria control measures and general improvement in health of the community [9,10]. On the southernmost island of Aneityum, a comprehensive elimination program was initiated in 1991 and elimination was achieved with a high degree of commitment from the local community in 1999 [11]. The Aneityum Project served as a proof of principle for the intensification of the malaria control program with the ultimate goal of elimination [9,10].

Previous population genetics studies of *P. falciparum* and the malaria vector *Anopheles farauti s.s.* in Vanuatu showed that populations were largely isolated on individual islands, with little gene flow among islands [12,13]. These findings implied that malaria control measures might be carried out on an island-by-island basis, which is the strategy currently used in the Pacific [10].

Merozoite surface protein 1 (MSP1) and circumsporozoite protein (CSP) are major surface antigens in *P. falciparum* and *P vivax*. These antigens are highly polymorphic, making them useful markers for assessment of parasite genetic diversity [7]. Earlier we examined *msp1* and *csp* polymorphisms in parasites from Vanuatu in the context of vaccine development for *P. falciparum* [14] and persisting humoral immunity after elimination on Aneityum Island for *P. vivax* [5]. In this study, using *msp1* and *csp* data previously generated for other aspects of malaria control, we compared the patterns of gene flow and population genetic structures in *P. falciparum* and *P. vivax* from seven sites on five islands in Vanuatu, and discussed the implications of our results in relation to the current malaria elimination strategy.

Materials and Methods

Ethics Statement

This study was approved by the Ministry of Health in Vanuatu and the Ethical Research Committee of Karolinska Institutet in Sweden. Due to the lack of a standardized writing system for the local "kastom" languages in Vanuatu, verbal informed consent was obtained from all adult participants and legal guardians in the case of minors. All pertinent information about the study, including the purpose, procedures, risks, benefits, and alternatives to participation, was provided to potential participants in both Bislama (lingua franca in Vanuatu; understood by most school-aged children and adults) by AK and the "kastom" language (understood by all participants) by local interpreters. The consent procedure was witnessed by a third party (e.g. teacher, village chief, nurse from local dispensary), who also recorded the name of each

participant as he/she enrolled in the study. The Ministry of Health in Vanuatu and the Ethical Research Committee of Karolinska Institutet in Sweden approved the use of this consent procedure.

Sample collections

P. falciparum and *P. vivax* isolates were collected during malariometric surveys conducted at seven sites on five islands (Gaua, Santo, Pentecost, Malakula, and Tanna) from five provinces in Vanuatu between 1996 and 2002 [5,14] (Fig. 1). Finger-pricked blood samples were collected on Whatman 31ET Chr filter paper (Whatman, Maidstone, UK) and stored desiccated [5].

DNA extractions, PCR amplifications, and genotyping/sequences

A subset of microscopy-positive samples from each site was randomly selected for this study. Genomic DNA was extracted from blood spotted on filter paper using the QIAamp DNA Blood Mini Kit (QIAgen, Germantown, MD) according to the manufacturer's instructions. PCR amplifications, and genotyping and/or sequencing of the merozoite surface protein 1 (*msp1*) and the circumsporozoite protein (*csp*) genes in *P. falciparum* [14,15] and *P. vivax* [5,16] were described previously. For each locus, samples with multiple alleles or genotypes were excluded for molecular analyses. The sequences described in this study have been deposited in the GenBank database (accession numbers AB116596-AB116607, AB539022-AB539045, and AB539540-AB539553).

Molecular analyses

For each locus, unbiased haplotype diversity (*H*) for each site was calculated using the equation $H = n(1 - \sum X_i^2)/(n-1)$, where n is the number of haplotypes and X_i is the frequency of the i-th haplotype [17].

Gene flow among populations was examined at two different levels. First, gene flow was examined among the seven sites. Second, populations from the same islands were pooled and gene flow was examined among the five islands.

For each locus, pairwise F_{ST} genetic distances among sites were estimated using the program Arlequin 3.5 [18]. Genetic distances for *P. falciparum* and *P. vivax* were based on the frequencies of shared haplotypes defined by sequence polymorphisms. The statistical significance of F_{ST} distances was evaluated by randomly permuting haplotypes between sites approximately 10,000 times to generate a null distribution against which the observed value was compared. Gene flow between sites was inferred when the pairwise F_{ST} genetic distance was not statistically significant ($p > 0.05$).

Genetic variation partitioned within populations, between populations within islands, and among islands was estimated by analysis of molecular variance (AMOVA) using the program Arlequin 3.5 [18]. The statistical significance of the observed values was evaluated by randomly permuting sequences among sites approximately 1,000 times to generate a null distribution against which the observed values were compared.

Results

P. falciparum and *P. vivax* infections

Overall, PCR amplifications of *msp1* and *csp* revealed more *P. falciparum* infections among the seven sites in Vanuatu (Table 1). *P. falciparum* was the predominant species in our samples from Pentecost and Malakula (Table 1). Different PCR efficacies between the *msp1* and the *csp* amplifications were likely the cause for the slightly different numbers of infections detected in

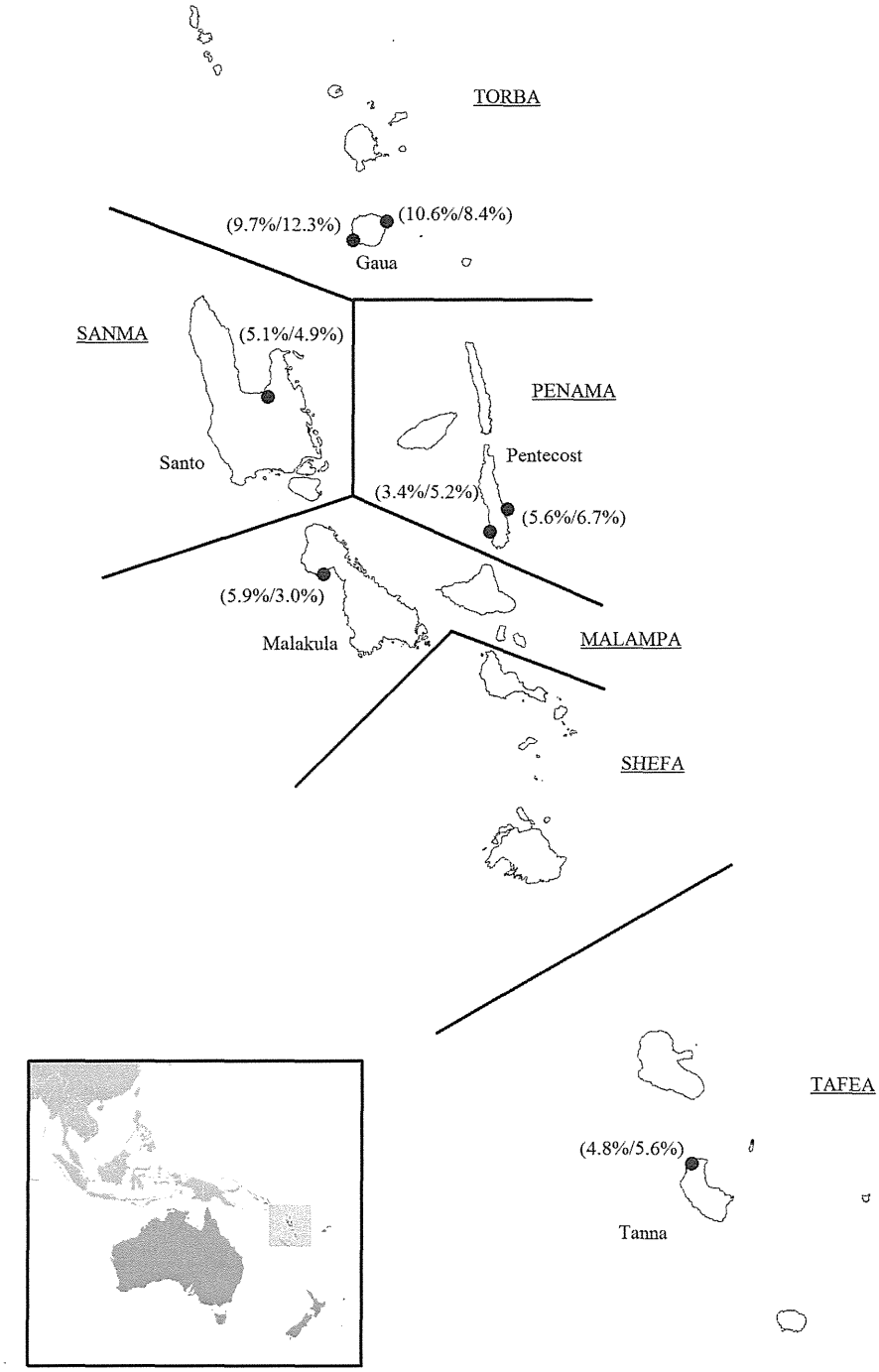

Fig 1. Map of Vanuatu showing the seven collection sites (black circles) on five islands. The names of the six provinces in Vanuatu are capitalized and underlined, and approximate provincial boundaries are indicated by solid lines. Species-specific parasite rates (*P. falciparum*/*P. vivax*) for each site were determined by microscopy. Maps were provided by the Library at the CIA (regional) and DIVA-GIS (Vanuatu).

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each site (Table 1). Multiple-genotype infections were more common in *P. vivax* than *P. falciparum* for both *msp1* (13.8% vs. 4.1%) and *csp* (36.2% vs. 3.6%) (Table 1).

Table 1. Numbers of merozoite surface protein 1 (*msp1*) and circumsporozoite protein (*csp*) sequences from seven sites in Vanuatu.

Site	*msp1* *P. falciparum*	*msp1* *P. vivax*	*csp* *P. falciparum*	*csp* *P. vivax*
East Gaua	16 (3)	23 (1)	19 (0)	14 (8)
West Gaua	14 (0)	10 (3)	10 (0)	6 (7)
Santo	24 (2)	27 (4)	21 (0)	23 (6)
East Pentecost	25 (1)	12 (2)	26 (0)	10 (9)
West Pentecost	16 (0)	3 (2)	14 (0)	2 (4)
Malakula	62 (1)	14 (4)	61 (6)	12 (7)
Tanna	8 (0)	11 (0)	8 (0)	7 (1)
Total	165 (7)	100 (16)	159 (6)	74 (42)

The numbers of infection with multiple genotypes are given in parentheses.

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P. falciparum and *P. vivax* genetic diversities

Genotyping and sequencing of *msp1* and *csp* revealed that *P. vivax* was more genetically diverse than *P. falciparum* in our samples from Vanuatu. In *P. falciparum*, six *msp1* and five *csp* haplotypes were observed, whereas in *P. vivax* 14 *msp1* and 20 *csp* haplotypes were observed (Tables A-D in S1 File). All *P. falciparum* isolates from Tanna (n = 8) were genetically identical for both *msp1* and *csp* (Tables A and B in S1 File). In *P. falciparum*, *msp1* diversities ranged from 0 in Tanna to 0.692 in West Gaua, while *csp* diversities ranged from 0 in East Pentecost and Tanna to 0.733 in West Gaua (Table 2). Very few *P. vivax* isolates were successfully genotyped in West Pentecost (Table 1), resulting in the extreme difference in diversity estimates between the two loci (0 for *msp1* vs. 1 for *csp*; Table 2). Excluding this site, *msp1* diversities ranged from 0.697 in East Pentecost to 0.889 in West Gaua, while *csp* diversities ranged from 0.822 in East Pentecost to 0.952 in Tanna (Table 2). Haplotype diversities were significantly higher in *P. vivax* than *P. falciparum* for both *msp1* (*t*-test; $p = 0.0135$) and *csp* ($p = 0.004$) when *P. vivax* from West Pentecost was excluded for comparison.

Patterns of gene flow

Seven-site analyses. Analyses of F_{ST} genetic distances showed that gene flow among *P. falciparum* populations was restricted. Between populations, gene flow in *msp1* was limited to those from the same islands (Gaua and Pentecost; Table 3), while gene flow in *csp* was observed between the two populations on Gaua, between West Gaua and Malakula, and between Tanna and the two populations on Pentecost (Table 3).

Table 2. Haplotype diversities of *msp1* and *csp* in *P. falciparum* and *P. vivax* from seven sites in Vanuatu.

Site	*msp1* *P. falciparum*	*msp1* *P. vivax*	*csp* *P. falciparum*	*csp* *P. vivax*
East Gaua	0.6583	0.8854	0.5906	0.8791
West Gaua	0.6923	0.8889	0.7333	0.8667
Santo	0.6703	0.8234	0.5286	0.9012
East Pentecost	0.5133	0.6970	0.0000	0.8222
West Pentecost	0.4583	0.0000	0.3626	1.0000
Malakula	0.5600	0.8791	0.7098	0.8788
Tanna	0.0000	0.7091	0.0000	0.9524

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Table 3. Pairwise F_{ST} genetic distances based on *msp1* (lower triangle) and *csp* (upper triangle) haplotype frequencies in *P. falciparum* from seven sites in Vanuatu.

Site	East Gaua	West Gaua	Santo	East Pentecost	West Pentecost	Malakula	Tanna
East Gaua		–0.001*	0.100	0.614	0.346	0.188	0.463
West Gaua	–0.065*		0.160	0.647	0.301	0.055*	0.440
Santo	0.308	0.288		0.759	0.509	0.275	0.646
East Pentecost	0.193	0.217	0.386		0.240	0.327	0.000*
West Pentecost	0.338	0.340	0.391	0.068*		0.133	0.088*
Malakula	0.106	0.142	0.324	0.067	0.251		0.251
Tanna	0.591	0.585	0.209	0.645	0.708	0.512	

Gene flow as defined by non-statistically significant ($p > 0.05$) F_{ST} distance is indicated by an asterisk (*).

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In contrast, a greater degree gene flow among *P. vivax* populations was observed. Examination of the *msp1* F_{ST} distances revealed gene flow among all *P. vivax* populations in northern and central Vanuatu except those between East Gaua and West Pentecost (Table 4). The population in Tanna remained genetically distinct from all other populations, however (Table 4). For *csp*, gene flow was observed among all populations on Santo, Pentecost, Malakula, and Tanna. The two populations on Gaua were genetically distinct from those in Santo and East Pentecost. Also, East Gaua was genetically distinct from Malakula, while West Gaua was distinct from Tanna (Table 4).

Five-island analyses. Analyses of F_{ST} distances among islands revealed patterns of gene flow consistent with those from the seven-site analyses. For *P. falciparum*, gene flow among populations on different islands was very minimal. *P. falciparum* populations in central Vanuatu (Santo, Pentecost, and Malakula) were significantly differentiated from one another, despite relatively small distances separating these islands. Gene flow between Pentecost and Tanna was observed as a result of the significant sharing of the 42NE haplotype in *csp* (Fig. 2; Table B in S1 File). For *P. vivax*, gene flow among populations on different islands was more widespread. Gene flow among islands in central Vanuatu (Santo, Malakula, and Pentecost) was evident in both *msp1* and *csp* (Fig. 2). However, gene flow between the peripheral islands of Gaua and Tanna and the central islands was more limited. For Gaua, gene flow with Santo and Malakula was observed for *msp1* only, while for Tanna, gene flow with all other islands was observed for *csp* only (Fig. 2).

Table 4. Pairwise F_{ST} genetic distances based on *msp1* (lower triangle) and *csp* (upper triangle) haplotype frequencies in *P. vivax* from seven sites in Vanuatu.

Site	East Gaua	West Gaua	Santo	East Pentecost	West Pentecost	Malakula	Tanna
East Gaua		0.034*	0.075	0.123	0.056*	0.094	0.017*
West Gaua	–0.005*		0.073	0.129	0.094*	0.089*	0.089
Santo	0.014*	0.011*		–0.004*	0.008*	0.001*	0.028*
East Pentecost	0.056*	0.041*	–0.029*		0.132*	0.045*	0.063*
West Pentecost	0.203	0.164*	0.210*	0.193*		–0.043*	-0.041*
Malakula	0.003*	–0.023*	–0.034*	–0.028*	0.166*		0.065*
Tanna	0.164	0.114	0.205	0.264	0.438	0.170	

Gene flow as defined by non-statistically significant ($p > 0.05$) F_{ST} distance is indicated by an asterisk (*).

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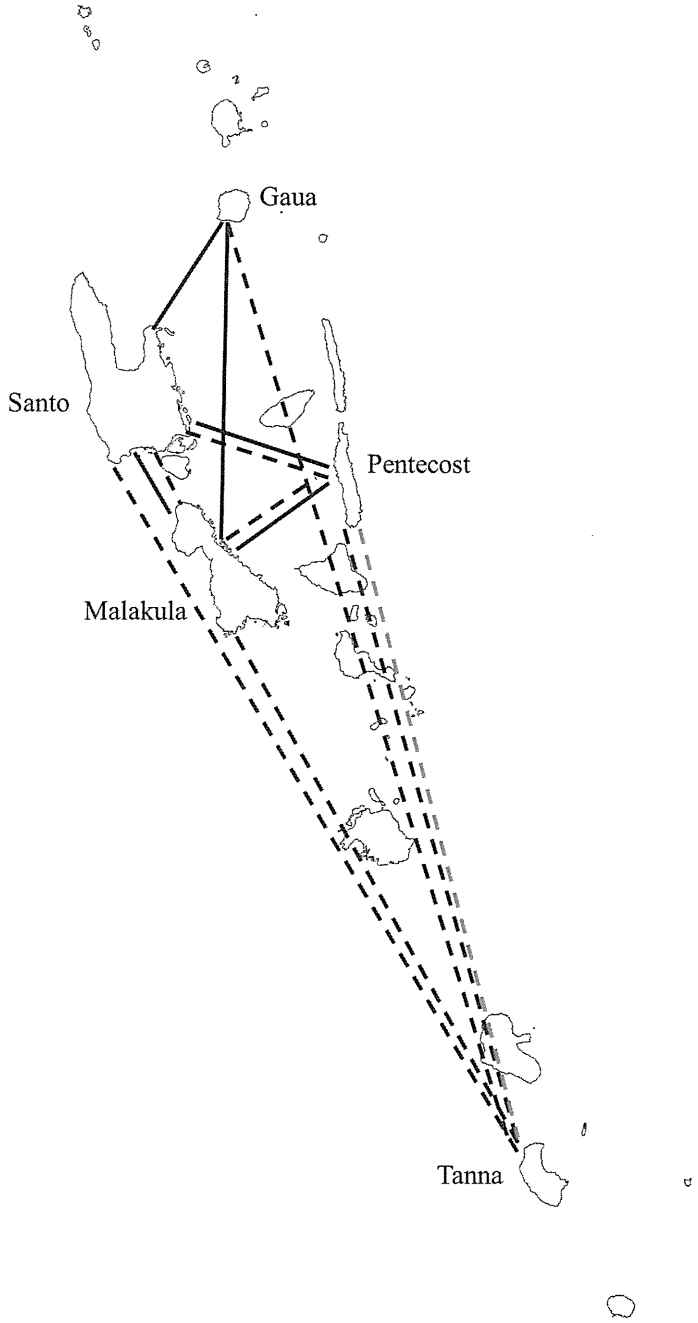

Fig 2. Gene flow among *P. falciparum* (gray) and *P. vivax* (black) populations from five islands in Vanuatu. Solid lines represent inferred gene flow based on merozoite surface protein 1 (*msp1*) F_{ST} genetic distances, while dotted lines represent gene flow based on circumsporozoite protein (*csp*) distances. No gene flow was observed in *P. falciparum msp1*. The map of Vanuatu was provided by DIVA-GIS.

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Partitioning of genetic variation

Different population genetic structures for *P. falciparum* and *P. vivax* were revealed by AMOVA (Table 5). For *P. falciparum*, while most of the genetic variation (66.7–70.7%) was found within sites, variation among islands was substantial (28.8–31.2%) and statistically significant ($p = 0.014$ for *msp1* and $p = 0.028$ for *csp*). This is consistent with the F_{ST} genetic

Table 5. Percentages of genetic variance partitioned at different population levels using analysis of molecular variance (AMOVA).

	P. falciparum	*P. falciparum*	*P. vivax*	*P.vivax*
Source of variation	*msp1*, %	*csp*, %	*msp1*, %	*csp*, %
Among islands	28.81*	31.23*	3.99	-0.53
Within island, between sites	0.53	2.06	2.93	5.77
Within sites	70.66	66.71	93.07	94.77

* $p < 0.05$

doi:10.1371/journal.pone.0119475.t005

distance analyses described above that showed significant genetic differentiation among most islands (Fig. 2). In contrast, almost all genetic variation (93.1–94.8%) in *P. vivax* was found within sites, and the lack of significant variation among islands ($p = 0.221$ for *msp1* and $p = 0.510$ for *csp*) was in agreement with gene flow among islands as revealed in the F_{ST} genetic distance analyses (Fig. 2).

Discussion

In Vanuatu, *P. falciparum* and *P. vivax* are the major malaria species, with a slight predominance of the latter [8]. In our samples we observed more *P. falciparum* than *P. vivax* infections, especially on Pentecost and Malakula (Table 1). Such difference might reflect the seasonal fluctuations in species prevalence in Vanuatu. Malaria transmission in Vanuatu is perennial. While incidence of *P. vivax* shows little seasonal fluctuation, incidence of *P. falciparum* peaks during the rainy season, from November to April [8].

Despite a slightly higher prevalence in our samples, *P. falciparum* showed consistently less genetic diversity than *P. vivax* in both *msp1* and *csp* across all sites except in West Pentecost, where few *P. vivax* isolates were found (Tables 1 and 2). Structural difference in orthologous genes between these two species may partially account for the difference in genetic diversity observed. For example, in *P. falciparum* sequence variation in *msp1* is dimorphic (either K1 or MAD 20 allelic type) and much of the variation is limited to the presence (or absence) and length of unique nine base-pair repeats in block 2 [19]. In contrast, *msp1* in *P. vivax* contains multiple variable blocks with extensive variation in repeats and nucleotide substitutions, and numerous potential recombination sites within and between variable blocks [20]. Direct comparison of genetic diversity in these orthologous loci between species may not be straightforward, nonetheless our result of lower genetic diversity in *P. falciparum* than in sympatric *P. vivax* was consistent with previous studies using neutral microsatellites [21,22] and other surface antigens such as apical membrane antigen 1 (*ama1*) [23,24].

Compared to *P. falciparum*, multiple-genotype infections were more common in *P. vivax* (Table 1). High frequencies of multiple-genotype infections facilitate meiotic recombination in the *Anopheles* mosquito vectors, leading to generation of novel genotypes [25] and greater genetic diversity in *P. vivax* (Table 2). In our *P. vivax* samples, the higher frequency of multiple-genotype infections in *csp* vs. *msp1* (36.2% vs. 13.8%; Table 1) was consistent with previous results from Thailand [26] and India [27].

Both MSP1 and CSP are major surface antigens, and high levels of polymorphisms in these loci are known to be a result of selection by host immunity [7,14]. However, it remains unclear whether the selective pressure on, and by extension genetic diversity in the orthologous loci of *P. falciparum* and *P. vivax* are directly comparable [7]. Differential *msp1* and *csp* genetic diversity in our samples might reflect differential selection by host immunity, i.e. stronger immune selection on the *P. falciparum* orthologs reduced genetic diversity observed in our samples.

Moreover, specific host immune response to MSP1 and CSP may differ between *P. falciparum* and *P. vivax*, resulting in different patterns of selection seen among the orthologs [28,29]. It has been shown that in *P. vivax*, rapid expansion and contraction of repeats in *csp* by slipped-strand mispairing was driven by immune selection [29], consistent with our observation of related and near-identical *csp* haploptes (e.g. VC06/13/16/19; Table D in S1 File) and high frequencies of *csp* multiple-genotype infections (Table 1).

At the global level different evolutionary histories of *P. falciparum* and *P. vivax* likely contributed to the high level of genetic diversity seen in the latter [30], however in Vanuatu the role of population history in shaping parasite genetic diversity is not well understood. *P. vivax* is believed to have accompanied *Homo sapiens* when the latter first settled the Pacific > 40,000 years before present (ybp), compared to the relatively recent arrival of *P. falciparum* within the last 10,000 years [31–33]. However, northern Vanuatu was first settled only 3200 ybp by Lapita migrants from the Solomon Islands [34], suggesting that both *P. falciparum* and *P. vivax* were introduced to Vanuatu at the same time [8,31,32]. Despite similar time depth within Vanuatu, the founder effect associated with the initial colonization might have been different between the two parasite species. Previous studies on *P. falciparum* and *P. vivax* population genetics showed a decrease in *P. falciparum* microsatellite genetic diversity in Temotu Province of the Solomon Islands when compared to Papua New Guinea, but no decrease in *P. vivax* [21,35], suggesting that the effective (reproductive) population sizes of the founding populations in the Solomon Islands might have been different between these two parasite species. To the south-east of the Solomon Islands, the initial introduction of malaria parasites to Vanuatu represents yet another founding event. Our observation of lower genetic diversity in *P. falciparum* from Vanuatu is consistent with the results from Temotu Province in the Solomon Islands [21], which was also first settled by Lapita migrants about 3200 ybp [36], further supporting the idea that genetic drift (founder effect) might have played a greater role in shaping the genetic diversity of *P. falciparum* than that of *P. vivax* in Vanuatu.

In Vanuatu, inter-island gene flow likely contributed to the higher genetic diversity in *P. vivax* populations in two ways. First, gene flow mitigates the loss of haplotypes due to genetic drift in isolated island populations. In *P. vivax*, the most abundant *msp1* and *csp* haplotypes were shared among all five sampled islands, whereas in *P. falciparum* no *msp1* and *csp* haplotypes were shared by more than three and four islands, respectively (Tables A and B in S1 File). Second, maintenance of distinct haplotypes in a population allows for generation of novel haplotypes by recombination. For example, in *P. vivax msp1* haplotypes VM03 and VM08 might have arisen from a recombination event between haplotypes VM01 and VM06 on Malakula, where all four lineages were found (Table C in S1 File). Recombination within the poly-Q sequence in block 6 of *msp1* might have further enhanced the polymorphic nature of the gene in *P. vivax* (Table C in S1 File). In contrast, limited recombination events [19] as a result of isolation shown here and previously [12] might have contributed to the relatively lower level of genetic diversity observed in *P. falciparum*.

Gene flow in *P. vivax* might be facilitated by its ability to form dormant hypnozoites in the host liver and the rapid development and emergence of gametocytes. Anti-hypnozoite treatment with primaquine is not usually administered to local *P. vivax* cases in Vanuatu [5]. Furthermore, unlike those with blood-stage parasites, *P. vivax* hypnozoite-carriers are asymptomatic and might therefore be less averse to long-distance travel (e.g. between islands). Once activated, latent hypnozoites develop into merozoites, which invade red blood cells to start the erythrocytic cycle of infection. In contrast to *P. falciparum*, *P. vivax* gametocytes are known to develop early, often before symptoms appear and treatments are sought, making *P. vivax* transmission efficient and persistent [37,38]. The period of extrinsic development of *P. vivax* is known to be shorter than that of *P. falciparum* [39], which may further facilitate *P. vivax*

transmission. In Vanuatu, *An. farauti s.s.* is the sole malaria vector [13]. It is unknown whether the efficiency with which this vector transmits parasites is different between *P. falciparum* and *P. vivax*, and how this difference, if it exists, might affect gene flow and genetic diversity.

Even though *P. vivax* showed a greater degree of gene flow, the extent of parasite movement appears to be distance dependent. Gene flow among *P. vivax* populations from the central islands of Santo, Malakula, and Pentecost was observed for both *msp1* and *csp*, while populations from the peripheral islands of Gaua and Tanna were more isolated, showing gene flow with these central island populations in only one locus. Parasite movement among Santo, Malakula, Pentecost, and to a less extent Gaua, is consistent with the existence of traditional exchange networks in northern and central Vanuatu, where both cultural (e.g. shell, pottery, mats) and biological (e.g. kava, yams, pigs) items are transported and traded across many islands [40]. It is reasonable to hypothesize that *P. vivax* is also transported and exchanged among these islands, albeit unintentionally. Tanna is not known to be a part of the aforementioned traditional exchange networks, instead parasite movement and gene flow between Tanna and these other islands might reflect the recent convenience of interisland air travel [9].

As samples used in this study were collected over a span of six years (1996 to 2002), potential temporal variation in parasite populations should be considered in the interpretation of parasite genetic diversity and gene flow. We evaluated the temporal "stability" of parasite populations from four sites (Santo, Malakula, East and West Pentecost) in which there were samples from at least two years. Analyses of *msp1* and *csp* F_{ST} genetic distances revealed no year-to-year differentiation among *P. vivax* populations from the same site, indicating that *P. vivax* populations remained relatively stable over the sampled period. For *P. falciparum*, genetic differentiation was observed among temporal populations from Malakula (both *msp1* and *csp*) and East Pentecost (*msp1* only). Given that *P. falciparum* incidence in Vanuatu shows strong seasonality [8], drastic year-to-year changes in the genetic makeup of *P. falciparum* populations due to genetic drift during the dry season are not unexpected. More comprehensive sampling of contemporaneous parasite populations from different islands will allow for a more refined description of gene flow in both *P. falciparum* and *P. vivax*.

Distinct parasite population structures and patterns of gene flow between *P. falciparum* and *P. vivax* have important implications on the current malaria initiatives in Vanuatu. Our previous analyses of *P. falciparum* and *An. farauti s.s.* genetic diversities showed that these two species were largely localized to individual islands [12,13]. However for *P. vivax*, we demonstrated that parasite movement among islands and across provincial boundaries is common, suggesting that the current island-by-island elimination strategy might need to be complemented with more integrated control and coordination among islands and provinces [10]. Moreover, the risk of resurgence or reintroduction of parasites from other islands after elimination should not be underestimated, as shown by our own experience on Aneityum Island, where *P. vivax* from Tanna was responsible for the outbreak six years after initial elimination [5].

Conclusions

In Vanuatu, *P. falciparum* and *P. vivax* were both present but showed different levels of genetic diversity and different patterns of gene flow and population structures. The high level of diversity in *P. vivax* populations was maintained by greater degree of gene flow among islands, which also resulted in greater genetic similarity among populations on different islands. Our data suggested that the current malaria control strategy might need to be bolstered with centrally integrated components and coordination among islands and provinces to ensure elimination and sustainable malaria freedom.