Mosquito Behavior Change After Distribution of Bednets Results in Decreased Protection Against Malaria Exposure

Edward K. Thomsen, Gussy Koimbu, Justin Pulford, Sharon Jamea-Maiasa, Yangta Ura, John B. Keven, Peter M. Siba, Ivo Mueller, Manuel W. Hetzel, and Lisa J. Reimer

1Liverpool School of Tropical Medicine, United Kingdom; 2Papua New Guinea Institute of Medical Research, Madang; 3Papua New Guinea Institute of Medical Research, Goroka; 4Michigan State University, East Lansing; 5Walter and Eliza Hall Institute, Parkville, Victoria, Australia; 6Swiss Tropical and Public Health Institute, Basel, Switzerland; and 7University of Basel, Switzerland

**Background.** Behavioral resilience in mosquitoes poses a significant challenge to mosquito control. Although behavior changes in anopheline vectors have been reported over the last decade, there are no empirical data to suggest they compromise the efficacy of vector control in reducing malaria transmission.

**Methods.** In this study, we quantified human exposure to both bites and infective bites of a major malaria vector in Papua New Guinea over the course of 4 years surrounding nationwide bednet distribution. We also quantified malaria infection prevalence in the human population during the same time period.

**Results.** We observed a shift in mosquito biting to earlier hours of the evening, before individuals are indoors and protected by bednets, followed by a return to preintervention biting rates. As a result, net users and non–net users experienced higher levels of transmission than before the intervention. The personal protection provided by a bednet decreased over the study period and was lowest in the adult population, who may be an important reservoir for transmission. Malaria prevalence decreased in only 1 of 3 study villages after the distribution.

**Discussion.** This study highlights the necessity of validating and deploying vector control measures targeting outdoor exposure to control and eliminate malaria.

**Keywords.** Infectious Disease Vectors; Mosquito Control; Mosquito behavior; Insecticide-Treated Bednets; Malaria.

In the past 2 decades, global efforts to reduce the burden of malaria have intensified. Since 2000, the primary strategy to limit transmission has been the distribution of insecticide-treated bednets (ITNs). Recent estimates suggest that in the last 15 years, ITNs have been responsible for preventing 68% of the 663 million cases that have been averted in sub-Saharan Africa due to increased malaria control efforts [1]. However, it is well recognized that strategies solely targeting endophagic, anthropophagic, and endophilic vectors may not be sufficient to control and eliminate malaria [2]. This is particularly true outside Africa where vectors exhibit greater behavioral plasticity. Control efforts can result in shifts in vector behavior and/or species composition such that the post-intervention vector community is less likely to come in contact with insecticide [3–9].

Studies to accurately quantify exposure to bites and the true protective efficacy of long-lasting insecticidal nets (LLINs) [10] have revealed that the vast majority of exposure still occurs inside, when people can be protected by an LLIN [11]. Thus, despite shifts to outdoor feeding [4, 5, 12] and changes in biting times [13], the personal protection provided by LLINs remains high (>80%). In areas outside of sub-Saharan Africa where vectors bite earlier and outside, LLINs can still reduce transmission through the combined effect of frequent blood-feeding and a homogenous host-seeking phenotype [14, 15]. Regardless, in some settings, evidence suggests that these behavioral changes are decreasing the personal protection against bites offered by an LLIN [16], a worrying prospect for malaria control and elimination in these areas.

It is well established that residual malaria transmission (transmission that remains despite universal coverage of effective interventions [2]), can be intense. However, it is currently unknown whether the interventions that are deployed against malaria vectors have the ability to increase residual transmission intensity through shifts in behavior or how shifts in behavior may impact human infection prevalence. Modeling suggests that the presence of behavioral resistance could dramatically increase transmission, perhaps more so than physiological resistance [8], which is currently poised to create a public health disaster if not confronted immediately. Behavioral resistance could have catastrophic consequences for the sustainability of currently available vector control methods, especially in areas outside of sub-Saharan Africa characterized by outdoor transmission [17, 18]. In this study, we estimate the exposure
mosquito controlled by insecticide-treated nets (ITNs). The authors of this study provide a valuable insight into the impact of ITNs on malaria prevalence and biting behavior, suggesting that while ITNs can significantly reduce malaria transmission, their protective efficacy may decrease over time due to factors such as net deterioration and behavioral changes in the population. The study also highlights the importance of continuous monitoring and intervention strategies to maintain the effectiveness of ITNs in malaria control.

To estimate the proportion of bites experienced inside and outside, additional collections were performed in June 2011. During this month, indoor landing catches were performed simultaneously with the outdoor landing catches at 1 chosen household per night for 6 consecutive nights. The degree of endophagy is presented as the proportion of mosquitoes collected by indoor landing catches out of the paired total.

**Human Behavior**

Human movement inside and outside was quantified as part of a national household survey and questionnaire [19]. Heads of household were asked what time individuals in the house went inside, what time they went to bed, and how old they were. Kokofine and Mauno were not included as part of this household survey have already been described [19]. Briefly, a finger prick blood sample was taken from consenting individuals aged ≥5 months old from 30–35 randomly selected households in each village. Stained blood slides were double-read by trained microscopists at the PNG Institute of Medical Research.

**Data Analysis**

Nightly biting rates were compared between years using a 1-way analysis of variance and Tukey’s test for post-hoc comparisons. Median biting times, 1st and 3rd quartiles, and 95% confidence intervals were calculated based on the entire catch per village and year. Kruskal–Wallis tests with pairwise comparisons were performed to determine whether the distribution of biting times between years was the same. Sporozoite prevalence was calculated as minimum prevalence, where positive pools were assumed to only have 1 positive mosquito. Prevalence for each year was calculated by dividing the total number of positive pools (with either *P. falciparum* or *P. vivax*) by the total number of mosquitoes in all pools analyzed. Prevalence was compared between years with chi-square tests. At 10 PM, 90% of individuals were inside, so this time point was chosen to compare sporozoite prevalence in early biting mosquitoes using a chi-square test. Four indices of exposure and protection were estimated: exposure to bites (either for a net user [B\_u] or a non–net user [B\_n]), the proportion of exposure occurring indoors (π\_i), the true protection against mosquito bites (P\_t), and the true protection against infective bites (P\_t^\*). Estimates of exposure to bites for net users and nonusers were calculated as published previously [10] with 2 modifications. First, because paired indoor and outdoor landing catches were not performed during the entire study period, indoor hourly biting rates were estimated by first calculating the hourly proportions biting inside and outside during the paired collections. Hourly proportions were then multiplied by hourly outdoor biting rates to estimate hourly indoor biting rates. Second, estimates of indoor exposure for net users was refined by accounting for the period after individuals moved inside and before they went to bed. The estimate of exposure for a net user (B\_u) was therefore:

\[
B_u = \sum_{t=1}^{24} \left[ B_{o,t} (1 - I_t) + B_{i,t} (I_t - S_t) + B_{o,t} S_t (1 - P) \right],
\]

where B\_o,t is the outdoor biting rate at time t, I\_t is the proportion of individuals inside at time t, B\_i,t is the indoor biting rate at time t, S\_t is the proportion of individuals sleeping at time t, and...
P is the protection provided by nets, which is assumed to be 0.968 [23]. Similar modifications were made to the calculation of \( B_u \), \( P^* \), and \( \pi_t \).

Exposure to infective bites was estimated by first calculating the hourly infection rate \( N_i \). Exposure to infective bites for a net-user was therefore

\[
F_p = \sum_{i=1}^{24} B_{p_i} N_i,
\]

and for a non-user

\[
F_u = \sum_{i=1}^{24} B_{u_i} N_i.
\]

The personal protection against infective bites \( (P^f) \) provided by an LLIN was

\[
P^f = 1 - \frac{F_u}{F_p}.
\]

\( B_u \) was compared across years in each village using Kruskal–Wallis tests with pairwise comparisons. \( \pi_t \) and \( P^* \) were compared among age groups and years using generalized linear mixed models with a binomial distribution, village as a subject, year by age group as the fixed effect, and household nested within date as a random effect. For each dependent variable, a dataset was constructed using the formulas described herein (or derivatives of) to estimate exposure values for each household and date combination. All statistical analyses were performed with SPSS 22.

Prevalence of malaria positivity was compared between years within each village using chi-square tests.

Ethical Approval and Informed Consent

Informed consent was obtained from all participants or their parent/guardian for those aged <16 years. This study was approved by the institutional review board at the Papua New Guinea Institute of Medical Research (protocol 0933) and the Medical Research Advisory Council of PNG (protocol 10.12).

Results

Biting Rates

Over the course of 4 years (2008–2011), 41 757 anopheline mosquitoes were captured by 138 outdoor human landing catch collections. More than 99% \((n = 41407)\) were identified as An. farauti sense lato. The remaining mosquitoes were identified as An. koliensis \((n = 157)\), An. punctulatus \((n = 122)\), An. longirostris \((n = 69)\), and An. karwari \((n = 2)\). All 4267 of the An. farauti s.l. mosquitoes that were confirmed by polymerase chain reaction were An. farauti 4. The nightly biting rate significantly decreased 1 year after LLINs were distributed in both villages (from 560 to 212 bites/person/night in Kokofine, \( P = .001; \)

and from 156 to 37 bites/person/night in Mauno, \( P < .001)\). In Kokofine, nightly biting rates increased significantly in 2010 (to 374 bites/person/night) and remained at that level in 2011 (418 bites/person/night). In Mauno, nightly biting rates remained low but did increase significantly between 2010 (4 bites/person/night) and 2011 (66 bites/person/night, \( P < .001)\).

Host-Seeking Behavior

The median outdoor biting time in both villages occurred significantly earlier after the distribution of LLINs (Figure 1A and 1B). In Kokofine, the median biting time was 11 PM–12AM in 2008 and was 1 hour earlier in 2009. Although the value of median biting time returned to 11 PM–12AM in 2010–2011, there was still a significant shift from the preintervention value due to the change in bite time distribution, with more mosquitoes biting earlier than the median time after LLIN distribution. In Mauno, the median biting time was 12 PM–1 AM in 2008, and shifted 2 hours earlier (10 PM –11 PM) in 2009. In 2010 and 2011, the median biting time remained at 10 PM–11 PM, but the distribution of bites continued to shift even earlier. In both villages, the hour of maximum biting density was 10 PM–11 PM in 2008, and 8 PM–9 PM in 2011 (Figure 1C and 1D). The degree of endophagy remained relatively consistent throughout the hours of the night, with 16.5% of overall bites occurring inside (Supplementary Figure 1).

Mosquito Infection Prevalence

Sporozoite prevalence remained consistent across all 4 years in Mauno but increased significantly in 2011 in Kokofine (Figure 2). Mosquito infection prevalence ranged from 0% to 0.54% in Kokofine and 0% to 0.42% in Mauno. The proportion of infective bites occurring before 10 PM was not different between years.

Human Sleeping Behavior

Movement of people indoors occurred slightly earlier in the highland than in the lowland regions of PNG. Sleeping patterns were similar across the 4 main geographical regions (Supplementary Figure 2).

in Momase, where Kokofine and Mauno are located, data were disaggregated by sex and age. Adolescent and adult males had later patterns of activity than females, and younger individuals went inside and went to bed earlier than older individuals (Figure 3A and 3B). The proportion of individuals sleeping under an LLIN did not exceed 0.71 among any age group at any time of night, with males aged 15–19 years the least protected at 0.54 (Figure 3C). A separate study in this village reported net usage at 91% in 2012 (J. Keven, L. Reimer, M. Katusele, G. Koimbu, R. Vinit, N. Vincent, E. Thomsen, D. Foran, P. Zimmerman, and E. Walker, submitted).

Exposure

After a significant decrease in exposure between 2008 and 2009 in both villages (\( P < .001 \) for Kokofine and Mauno),
there was subsequently a significant increase from 2009 to 2011 in Kokofine and from 2010 to 2011 in Mauno (Figure 4). Shifts to earlier bite exposure were observed in both villages (Supplementary Figure 3). Within each year, the estimated proportion of exposure occurring inside ($\pi_I$) and the protective efficacy against bites ($P^*$) was significantly greater in younger age groups ($P < .001$ for all years). Within each age group, there was a decrease in $\pi_I$ and $P^*$ after LLINs were distributed, and

---

**Figure 1.** Median outdoor biting times with 1st and 3rd quartiles (boxes) and 95th percentiles (whiskers) in Kokofine (A) and Mauno (B) villages of Papua New Guinea before (2008) and after (2009–2011) a long-lasting insecticidal net distribution. Years not sharing the same letter indicate significantly different medians using a Kruskal–Wallis test with pairwise comparisons. The proportion of bites occurring at each hour in Kokofine (C) and Mauno (D) are presented as well.

**Figure 2.** Sporozoite prevalence for *Plasmodium falciparum* and *Plasmodium vivax* in *An. farauti 4* in Kokofine (A) and Mauno (B) villages of Papua New Guinea before (2008) and after (2009–2011) a long-lasting insecticidal net distribution. Sample sizes are indicated below each year. Bars not sharing the same letter indicate significant differences using chi-square tests.
the decrease was more pronounced in younger age groups (Figure 5; for \( \pi_i \): <5, \( P < .001; 5–9, P = .002; 10–14, P = .004; 15–19, P = .008; >20, P = .03; \) and for \( P^* \): <5, \( P = .001; 5–9, P = .003; 10–14, P = .006; 15–19, P = .01; >20, P = .02). In Kokofine, the rebound in biting rates coupled with high sporozoite prevalence after LLIN distribution (in 2011) allowed us to quantify exposure to infective bites (Figure 6). In 2008, most exposure to infective bites occurred after 9 PM. In 2011, the majority of infective bites occurred during the first hour of collection, between 6 PM and 7 PM. In children aged <5 years, many infective bites would have been prevented by using a net in 2008; however, the protective efficacy of LLINs against infective bites (\( P^* \)) decreased drastically in 2011 because these bites were occurring before this age group went to bed. In adults aged >20 years, a similar but less pronounced decrease in \( P^* \) was observed, primarily because this age group was always outside when infective mosquitoes were seeking a host. In 2008, \( P^* \) was 0.78 in those aged <5 years and 0.30 in those aged >20 years. In 2011, \( P^* \) had decreased to 0.30 and 0.15 in both groups, respectively.

**Human Infection Prevalence**

Data from the 2008 and 2009 malaria prevalence surveys have been published elsewhere [19] and are presented in greater detail here for context. Only Mauno showed a consistent and significant decrease in malaria prevalence across the 3 surveys. In Kokofine, there was no significant change in overall infection prevalence, and in Kesowai there was a nearly significant increase in prevalence from 2008 to 2011 (\( P = .058 \)) (Figure 7).

**DISCUSSION**

Our data show a clear and dramatic reduction in mosquito abundance in the first year after the LLIN distribution in both villages. However, a resurgence in mosquito abundance and exposure was documented between 2 and 3 years after intervention, coupled with a shift to significantly earlier biting. Based on the interaction between mosquito and human behavior, the protective efficacy of LLINs decreased during this resurgence, as more exposure to malaria vectors occurred before individuals were protected with a net. Besides behavioral resilience [24], 2 other factors may have contributed to the documented resurgence in mosquito abundance. First, physiological resistance to insecticides has also been shown to reduce intervention efficacy [25]. However, resistance is absent in members of the *An. punctulatus* group (the species group to which *An. farauti* 4 belongs) in PNG [26], and susceptibility has been confirmed from the Sausi region post-LLIN distribution (M. Katusele, unpublished data). This demonstrates...
that behavioral resilience may compromise intervention efficacy in a mosquito population that is fully susceptible to insecticide. Second, changes in bednet usage over time could limit the community effect of nets; however, usage increased over the study period. Furthermore, used nets from local communities retain the insecticidal effect against *An. farauti* for 5 years [27].

This is the first study to quantify human malaria infection prevalence in the context of shifting mosquito behaviors after an LLIN distribution. Malaria prevalence in humans decreased in only 1 of 3 villages, the village with the lowest biting rates, demonstrating the limited efficacy of nets to prevent disease transmission. Although the shifts in biting times would contribute to the limited epidemiological impact of the intervention, there are other factors that may have also played a role. Artemisinin combination therapy was only rolled out to the Sausi health center in the last quarter of 2011, which means that the population may have been receiving inadequate treatment. Treatment failures with the previous combination of chloroquine and sulphadoxine-pyrimethamine reached 18.5% in children with *P. falciparum* in PNG [28]. Migration of individuals into the study communities from areas of higher malaria burden may have also been a factor. Regardless, shifts in biting times have been documented in other mosquito populations in PNG [9]. As such, it will be important to continually monitor the epidemiological impact of LLINs in other areas where changes in mosquito behavior have been observed.

This is also the first study to quantify age-stratified exposure to bites of malaria vectors by taking into account the behaviors and sleeping patterns of each age group. Both the proportion of indoor bite exposure ($r_i$) and the protective efficacy of LLINs against bites ($P^*$) is greater in younger age groups, due to earlier sleeping patterns. This results in protection by an LLIN for a greater proportion of the entire period of exposure, which is a positive finding because this group is the most at risk for severe disease [29]. In contrast, the protective efficacy in adults is quite low (approximately 0.35 in both villages at the time of distribution) due to their greater outdoor activity patterns in the early hours of the night. Adults will continue to act as a reservoir of gametocytes, and LLINs may therefore have little impact on transmission reduction.
of the *Plasmodium* extrinsic incubation period still remained high [15]. Unlike the studies described above, our study suggests that the shift to early evening feeding in *An. farauti* 4 is epidemiologically significant—the estimate of the annual entomological inoculation rate in Kokofine was 827 infective bites per person per year in 2011, more than double the estimate of 343 in 2008. In addition, our analysis indicated that individuals were less protected from infective bites in 2011 than they would have been in 2008 due to the time infective mosquitoes were collected.

The underlying mechanism for the shift in biting times observed in this vector population is currently unknown. Biting behavior in anophelines appears to be a heritable trait because shifts in host-seeking behavior in the Solomon Islands during the dichlorodiphenyltrichloroethane spray campaign of the 1970s [3] remain to this day [16]. However, additional evidence for population-level selection for behavioral resistance is lacking [7]. Today, populations of *An. farauti* s.s. in the Solomon Islands are homogenous in their host-seeking behavior: subpopulations exhibiting different feeding preferences do not exist [14]. In addition, the genes responsible for the variation in feeding behaviors in malaria vectors have yet to be identified [30]. Additional hypotheses for the mechanism include associative learning [24] and delayed host-seeking due to unsuccessful attempts the previous night [31].

The sampling scheme used in this study had several limitations. First, human landing catches were not performed before 6 pm. This may have resulted in significant undersampling of the biting population after LLIN distribution. Second, the ratio of indoor to outdoor biting rates was measured during 1 collection period after LLIN distribution. The high degree of exophagy measured here is consistent with reports of *An. farauti* 4 in neighboring Papua, Indonesia [18], as well as other members of the *An. farauti* complex in the Solomon Islands [32]. Decreases in endophagy have been observed following indoor interventions [3, 4], which we are unable to capture in our study design. If early biting mosquitoes were undersampled or if the population experienced a shift in endophagy, the analysis would have underestimated the decreases in personal protection. Third, collections were performed in 2 weeks in 2008, 2009, and 2010 and 6 weeks in 2011, which may have highlighted week-to-week variation and obscured long-term trends.

Indoor interventions such as LLINs have contributed greatly to the reduction in malaria transmission over the last 15 years [1] and continue to provide significant community protection even in cases where shifts in biting behavior have been observed [6, 11, 12, 14, 15]. Our study highlights that in an area of high vector density and intense year-round transmission, shifts in biting behavior can have detrimental impacts on the personal protection provided by LLINs as well as community-wide transmission. Shifts to earlier biting after the bednet distribution resulted in greater exposure to infective bites, in net users...
and nonusers alike. The intervention achieved a reduction in malaria prevalence in only 1 of 3 villages studied despite high usage rates and net efficacy. Additional tools targeting outdoor and early biting mosquitoes will be necessary to control malaria and prevent a resurgence of transmission.

Supplementary Data
Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
Acknowledgements. We acknowledge the Papua New Guinea Institute of Medical Research MalCon survey teams for the net use surveys and blood sample collection and Muker Sakur and Lemen Kilepak for assisting with mosquito collections. We would like to thank the communities in the Sausi region of Madang Province for participating in this study. We would also like to thank Dr Geraldine Foster and Professor Hilary Ranson for helpful comments on an early draft of the manuscript.

Financial support. This work was supported by the Global Fund to Fight AIDS, Tuberculosis and Malaria (grant number PNG-304-G01-M).

Potential conflict of interests. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References
1. Bhatt S, Weiss DJ, Cameron E, et al. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature 2015;1–9.
2. Killeen GF. Characterizing, controlling and eliminating residual malaria transmission. Malar J 2014;13:330.
3. Taylor B. Changes in the behaviour of a malaria vector, Anopheles farauti S., following use of DDT as a residual spray in houses in the British Solomon Islands Protectorate. Trans R Ent Soc 1975;127:277–292.
4. Russell TL, Govella NJ, Azizi S, Drakeley CJ, Kachur SP, Killeen GF. Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. Malar J 2011;10:80.
5. Reddy MR, Overgaard HJ, Abaga S, et al. Outdoor host seeking behaviour of Anopheles gambiae mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. Malar J 2011;10:184.
6. Moiroux N, Gomez MB, Pennetier C, et al. Changes in Anopheles funestus biting behavior following universal coverage of long-lasting insecticidal nets in Benin. J Infect Dis 2012;206:1622–9.
7. Govella NJ, Chaki PP, Killeen GF. Entomological surveillance of behavioural resilience and resistance in residual malaria vector populations. Malar J 2013;12:10–1186.
8. Gatton ML, Chitnis N, Churcher T, et al. The importance of mosquito behavioural adaptations to malaria control in Africa. Evolution 2013;67:1218–30.
9. Reimer LJ, Thomsen EK, Koimbu G, et al. Malaria transmission dynamics surrounding the first nationwide long-lasting insecticidal net distribution in Papua New Guinea. Malar J 2016;15:25.
10. Killeen GF, Kihonda J, Lymo E, et al. Quantifying behavioural interactions between humans and mosquitoes: evaluating the protective efficacy of insecticidal nets against malaria transmission in rural Tanzania. BMC Infect Dis 2006;6:161.
11. Huo B, Briet O, Seyoum A, et al. Consistently high baseline estimates for the proportion of human exposure to rural African malaria vector populations that occurred indoors. Malar J 2012;11:50.
12. Bradley J, Lines J, Fuscini G, et al. Outdoor biting by Anopheles mosquitoes on Bioko Island does not currently impact on malaria control. Malar J 2015;14:170.
13. Moiroux N, Damien GB, Egrot M, et al. Human exposure to early morning Anopheles funestus biting behavior and personal protection provided by long-lasting insecticidal nets. PLoS One 2014;9:e104967.
14. Russell TL, Beeve NW, Bugoro H, et al. Anopheles farauti is a homogeneous population that blood feeds early and outdoors in the Solomon Islands. Malar J 2016;15:151.
15. Russell TL, Beeve NW, Bugoro H, et al. Frequent blood feeding enables insecticide-treated nets to reduce transmission by mosquitoes that bite predominately outdoors. Malar J 2016;15:156.
16. Bugoro H, Cooper RD, Butafa C, et al. Bioinformatics of the malaria vector Anopheles farauti in Temotu Province, Solomon Islands: issues for malaria elimination. Malar J 2011;10:133.
17. Conn JE, Norris DE, Donnelly MJ, et al. Entomological monitoring and evaluation: diverse transmission settings of ICEMR projects will require local and regional malaria elimination strategies. Am J Trop Med Hyg 2015;93:28–41.
18. St Laurent B, Supratman S, Ashi PB, et al. Behaviour and molecular identification of Anopheles malaria vectors in Jayapura district, Papua province, Indonesia. Malar J 2016;15:192.
19. Hetzel MW, Reimer LJ, Gideon G, et al. Changes in malaria burden and transmission in sentinel sites after the roll-out of long-lasting insecticidal nets in Papua New Guinea. Parasit Vectors 2016;9:340.
20. Beeve 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;53:478–81.
21. Wirtz RA, Sattabongkot J, Hall T, Burket TR, Rosenberg R. Development and evaluation of an enzyme-linked immunosorbent assay for Plasmodium vivax VK2/47 sporozoites. J Med Entomol 1992;29:854–7.
22. Hetzel M, Pulford J, Gouda H, Hodge A, Siba P, Mueller I. The Papua New Guinea National Malaria Control Program: primary outcome and impact indicators, 2009–2014. Papua New Guinea Institute of Medical Research: Goroka, Papua New Guinea; 2014.
23. Ree H. Experimental hut studies on the effect of permethrin-treated mosquito-nets against Anopheles farauti in the Solomon Islands. World Health Organization: Geneva, Switzerland; 1988.
24. Killeen GF, Chitnis N. Potential causes and consequences of behavioural resilience and resistance in malaria vector populations: a mathematical modelling analysis. Malar J 2014;13:97.
25. N’Guessan R, Corbel V, Akogbéto M, Rowland M. Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area. Benin. Emerg Infect Dis 2007;13:199–206.
26. Keven JB, Henry-Haldin CN, Thomsen EK, et al. Pyrethroid susceptibility in natural populations of the Anopheles punctulatus group (Diptera: Culicidae) in Papua New Guinea. Am J Trop Med Hyg 2010;83:1259–61.
27. Katusele M, Gideon G, Thomsen EK, Siba PM, Hetzel MW, Reimer LJ. Long-lasting insecticidal nets remain efficacious after five years of use in Papua New Guinea. P N G Med J 2014;57:86–93.
28. Karunajeewa WA, Mueller I, Senn M, et al. A trial of combination antimalarial therapies in children from Papua New Guinea. N Engl J Med 2008;359:2545–57.
29. Genton B, D’Acremont V, Rare L, et al. Plasmodium vivax and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea. PLoS Med. 2008;5:881–9.
30. Maliti DV, Marsden CD, Main BJ, et al. Investigating associations between bitting time in the malaria vector Anopheles arabiensis Patton and single nucleotide polymorphisms in circadian clock genes: support for sub-structure among An. arabiensis in the Kilombero valley of Tanzania. Parasit Vectors 2016;9:109.
31. Charlwod JD, Dagoro H. Impregnated bed nets for the control of filariais transmited by Anopheles punctulatus in rural Papua New Guinea. P N G Med J 1987;30:199–202.
32. Bugoro H, Hii JL, Butafa C, et al. The bioinformatics of the malaria vector Anopheles farauti in Northern Guadalcanal, Solomon Islands: issues for successful vector control. Malar J 2014;13:56.
