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Review Article

# Possible New Controlling Measures for the Pyrethroid-Resistant Malaria Vectors

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**Abstract**

The dramatic success of long-lasting insecticidal nets (LLINs) in African countries has been countered by the rapid development of pyrethroid resistance in vector mosquitoes. The use of excito-repellency chemicals might be bio-rational, since such repellency will not induce physiological resistance. However, little is known about the relationship between the mode of insecticide resistance and excito-repellency in mosquitoes. The goals of our study were to investigate [1] the reactions of vector mosquitos in an area where pyrethroid resistance has developed, [2] the effect of LLINs on these malaria vectors, and [3] the development of new control techniques to supplement LLINs. Laboratory tests showed that resistant species governed by *kdr* (knockdown resistance) (*Anopheles gambiae s.s.*) lose repellency to pyrethroids, whereas those lacking *kdr* (*An. arabiensis* and *An. funestus s.s.*) maintain high repellency. LLINs were effective against these pyrethroid-resistant malaria vectors, because they limited feeding on humans during bedtime. However, notable time shifts in human blood feeding activity developed in both *An. arabiensis* and *An. funestus* s.s., whereas no such time shift developed in *An. gambiae s.s.* These time shifts might be partially explained by differences in repellency by pyrethroids for these species. LLINs might not be effective because most blood feeding occurs when people are active outside the bed nets. Screening eaves with pyrethroid-impregnated wide-mesh nets was found to be effective in reducing human exposure to malaria vectors. The excito-repellency of pyrethroids that act as a spatial barrier or reduce feeding motivation of mosquitoes might be another countermeasure.

## ABBREVIATIONS

LLIN: Long Lasting Insecticidal Net; ITN: Insecticide-Treated Net; ***kdr***: knock Down Resistance

## INTRODUCTION

The most important event in the history of mosquito control was the invention of dichloro-diphenyl-trichloroethane (DDT) by Dr. Paul Müller, who received the Nobel Prize for Chemistry in 1948. The long persistence and excellent killing efficacy of DDT as the agent in indoor residual spraying (IRS) are responsible for its brilliant success after the Second World War. Due to the failure of the malaria eradication program with DDT mainly because of mosquito resistance, the World Health Organization (WHO) has altered its policy from eradication to control by primary health care and the use of pyrethroid-impregnated mosquito nets. Global use of DDT, however, has not changed substantially since the Stockholm Convention came into force. India has dominated the global use of DDT (>80%), although it showed a modest decline in use after 2005, while in the African region, DDT use increased sharply until 2008 along with the expansion of IRS programs[1].

Research on the development of natural pesticides is considered to be a bio-rational approach because it may equate the adverse environmental effects of chemicals to those of naturally occurring substances. One of the most successful developments in the manufacturing of pesticide chemicals was the discovery of pyrethrum and the subsequent successful synthesis of pyrethroids. For example, one of the first synthesized pyrethroids, allethrin [2], continues to be used for preventing mosquito bites without any toxicological or operational problems. Devices with the highest popularity and the most long-standing use that incorporate pyrethroids are mosquito coils, mosquito mats, and liquid vaporizers. Pyrethroids belonging to the knockdown agent group, such as allethrin, pyrethrin, and prallethrin, are used in these devices.

The use of pyrethroids in insecticide-treated bed nets (ITNs) is a simple and inexpensive protection measure against malaria and has been shown to reduce morbidity in children less than 5 years old by 50% and global child mortality by 20–30% [3-5]. However, the impregnation and re-impregnation of ITNs requires technical skills, materials, and human labor, which may not always be available [6]. Mosquito nets pre-treated with insecticide were a breakthrough measure to resolve this problem [7]. Olyset® Net, made of polyethylene netting material (mesh 11 holes/cm$^2$) with permethrin (2%) incorporated into the polymer before monofilament yarn extrusion, and the PermaNet®, made of polyester netting material (mesh 25 holes/cm$^2$) with deltamethrin (55 mg ai/m$^2$) incorporated in a resin coating of the fibers, are two successful LLINs (long-lasting insecticidal nets) recommended by the WHO (http://www.who.int/whopes/Long_lasting_insecticidal_nets_06_Feb_2014.pdf).

In this review, some problems which barrier the effective control of malaria vector mosquitoes with LLINs, IRS, etc. are brought up and some possible countermeasures which might defeat the obstacles are proposed. Several attempts to develop new mosquito control techniques using a pyrethroid within the knockdown agent groups (metofluthrin) and a slow-released type I pyrethroid (permethrin) as a spatial and excito-repellent agent are also discussed.

## OPERATIONAL LIMITATIONS OF LLINS

ITNs or LLINs are effective against malaria vectors only when the vector mosquitoes are endophagous and their feeding time corresponds to the time when people are sleeping inside bed nets [8-10]. Behavioral changes in vector mosquitoes from endophagous to exophagous and/or the shifting of biting time from midnight to dawn or dusk may reduce their physiological resistance to insecticides as well as the effectiveness of bed nets. Our trial in residential houses in western Kenya where LLINs (Olyset® Nets) were properly used showed that the percentage of human feeding success was reduced to 15.9%–24.6% and concluded that LLINs were effective against three major malaria vectors (*An. gambiae* s.s., *An. arabiensis*, and *An. funestus* s.s.) during bedtime. The overall activity of the three primary malaria vectors in this study area has not changed from that reported prior to the extensive use of ITNs and LLINs in Africa. However, both *An. arabiensis* and *An. funestus* s.s. Had notable human blood feeding activity in the evening and slight activity in the early morning, while *An. gambiae* s.s. Did not, indicating that mosquito biting took place when people were active outside of the LLINs [9] (Figures 1,2).

Another limitation of the effectiveness of LLINs is the fact that they are only effective when people are inside of them. Recently, Iwashita et al. reported that bed net use by children between 5 and 15 years of age in villages along Lake Victoria in western Kenya was lower than that among other age classes. Bed net use was strongly affected by sleeping arrangement and the availability of suitable locations for hanging bed nets [10]. The ease of hanging a bed net is particularly important for children who often sleep in places such as living rooms where daily net hanging can be difficult and troublesome. Hence, the use of bed nets is sometimes limited to those sleeping in a bedroom (parents and babies). The rest of family members (in particular children older than 5 years) sleep in living rooms without a bed net, resulting in high numbers of *Plasmodium falciparum* cases in these age groups. Children who sleep on the floor are less likely to use LLINs, and *P. falciparum* infection was significantly higher among children who slept on the floor without LLINs [8] than among those who slept in beds with LLINs.

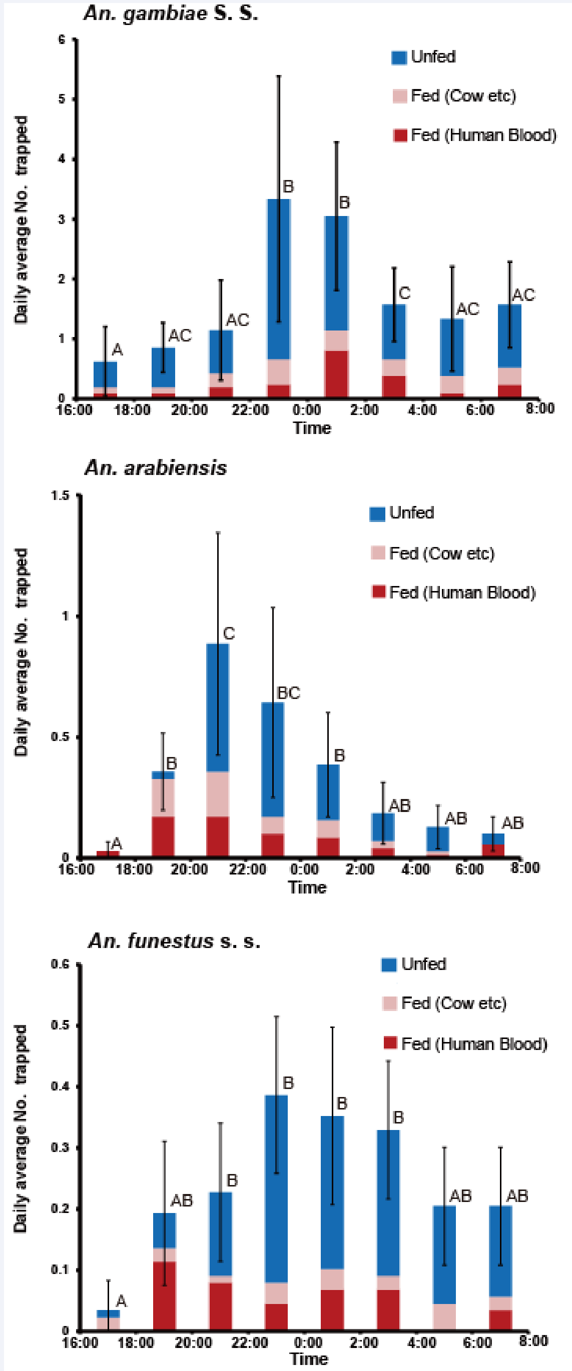


**Figure 1** Indoor activity pattern of female *Anopheles gambiae s.s.*, An. arabiensis, and An. funestus s.s. trapped by CDC miniature traps equipped with a collection bottle rotator in houses using LLINs (Olyset® Net). Indoor mosquito trapping was performed with 6 sets of the CDC miniature light trap (model 512) equipped with a collection bottle rotator (model 1512) (John W. Hock Co., FL, USA). Before the start of the study, each house was inspected and untreated bed nets and LLINs were exchanged for new permethrin-incorporated LLINs (Olyset® Nets). Additional new Olyset® Nets were provided for use in the living room when the residents (most were children >5 years) slept in the living room with no bed nets. Different letters indicate significant differences (Tukey's HSD test, $p < 0.05$), and bars indicate the 95% confidence limits for the total number of mosquitoes collected during each time period.

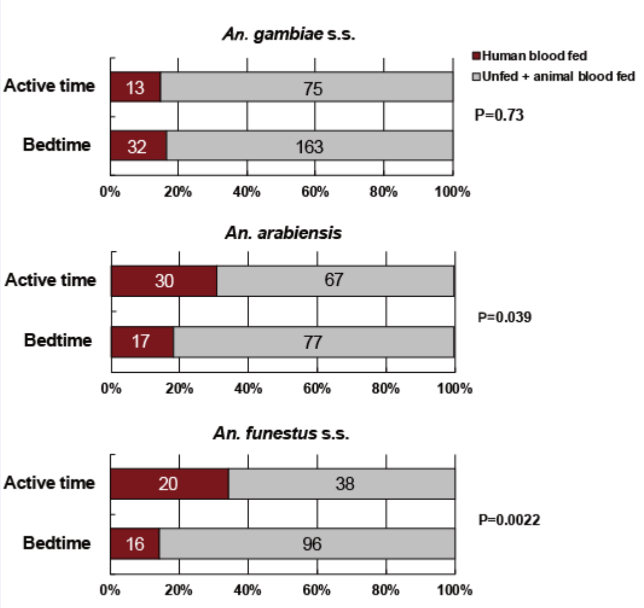


**Figure 2** The number of female mosquitoes collected at bedtime (10:00 PM–6:00 AM) and active time (4:00 PM–10:00 PM and 6:00 AM–8:00 AM) using a CDC miniature trap equipped with a collection bottle rotator.

Eaves (the gaps between the top of the wall and the roof) are common to houses throughout Africa and are thought to be the primary entrance for malaria vectors [11]. Changes in house design, such as the screening or closing of eaves, may reduce human exposure to malaria vectors [12]. Restructuring houses or physically closing the eaves will be expensive and may reduce the quality of living because of blocked ventilation.

The most important limiting factor of LLIN use is pyrethroid resistance in vector mosquitoes as well as insufficient population access to LLINs. Insecticide use continues to be the most effective means of controlling malaria, dengue hemorrhagic fever (DHF), and other arthropod-borne diseases (http://apps.who.int/iris/bitstream/10665/44670/1/9789241502153_eng.pdf). Globally, pyrethroids constitute approximately 81% of the spray utility, of which 68% is used for residual spraying and 24% for space spraying, and 100% of WHO-recommended insecticides for the treatment of LLINs [1]. Among pyrethroids that are used for vector control, 98.7% contain photo-stable pyrethroids such as α-cypermethrin, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, etofenprox, λ-cyhalothrin, and permethrin [13]. Pyrethroid resistance will be a major problem for the vector control program, since there are currently no suitable chemical substitutes for pyrethroids. Pyrethroid resistance is high in *An. gambiae* s.l. In West and Central Africa (file:///C:/Users/vergiss/Downloads/phe-atlas_final_version.pdf) [14]. Within Eastern and Austral Africa, *An. gambiae* s.l. Populations are mostly susceptible to pyrethroids in Tanzania [15], Mozambique [16], and Madagascar [17], but highly resistant in Uganda [18], Ethiopia [19], Kenya [20], Zambia [21], Zimbabwe [22], and South Africa [23]. The distribution of high allelic frequency of *kdr* (knockdown resistance) mutations (point mutations at the voltage-gated sodium channel, L1014S) in *An. gambiae* s.s. Converged in the northern and southern coastal regions as well as the western regions (including highland areas) of Lake Victoria [24]. These regions are some of the focal points identified as high vector transmission regions in Kenya, where more than 50% of the population is exposed to ≥ 40% PfPR2-10 (*P. falciparum* parasite rate corrected to a standard age-range of 2 to < 10 years old) [25] and there is high coverage by LLINs or ITNs. For example, the percentage of households with at least one LLIN in Nyanza and Western provinces (> 70%) was higher than that in the other provinces (< 70%) (Kenya HDS Final Report, 2009). Mathias et al. reported that the East African *kdr* allele (L1014S) concurrently increased in frequency during the past decade in *An. gambiae* s.s. (most of which are homozygous for the *kdr* allele) in western Kenya as household ownership of insecticide-treated bed nets increased regionally[26].

LLINs have several operational and critical limitations. Therefore, novel, convenient, and sustainable devices, or self-protection measures that can substitute or complement LLINs, are required for more effective prevention of malaria vectors.

## Effectiveness of metofluthrin-impregnated plastic strips as the spatial repellentsagainst vector mosquitoes

Metofluthrin, (Sumi One®) 2,3,5,6-tetrafluoro-4-methoxymethylbenzyl (E:Z ≈ 1:8)(1R, 3R)-2,2-dimethyl-3-(prop-1-enyl) cyclopropanecarboxylate, is a newly synthesized pyrethroid [27]. Metofluthrin belongs to the group of knockdown agents but has two unique characteristics that none of the conventional pyrethroids possess. Metofluthrin has a high vapor pressure ($1.87 \times 10^{-3}$ Pa at 25°C), which is 2-fold and 100-fold greater than that of *d*-allethrin and permethrin, respectively [27]. Metofluthrin vaporizes at room temperature, while other conventional pyrethroids require heating for vaporization. Another unique characteristic is its high killing efficacy against mosquitoes 28–79 times more effectively than *d*-allethrin [28]. These unique characteristics of metofluthrin may lead to the development of new mosquito controlling devices that do not require any external energy for vaporization and have lower costs and longer effective durations.

In preliminary studies, using a simple prototype device with metofluthrin-impregnated multilayer paper strips, the chemical showed promising spatial repellency against some mosquito species (*Anopheles sundaicus* (Rodenwaldt), *An. balabacensis* (Baisas), and *Culex quinquefasciatus* (Say) in both laboratory and field conditions on Lombok Island, Indonesia [29,30]. In order to increase the effectiveness of metofluthrin, Kawada et al., manufactured a cylindrical slow-release plastic device that was impregnated with 1000 mg metofluthrin in a 20 g strip [31]. High spatial repellent effect was observed in the beruga (a traditional wall-less outdoor living structure) that was treated with four metofluthrin-impregnated paper strips on the day of treatment. A significantly higher (>60%) spatial repellency ($p < 0.05$; Tukey's HSD test), than that observed in the untreated control, lasted for at least 11 wk with the two-strip treatment and for >15 wk with four-strip treatment (Figures 3,4).

The next step in the development of the devices was manufacturing a new plastic strip that would reduce the release

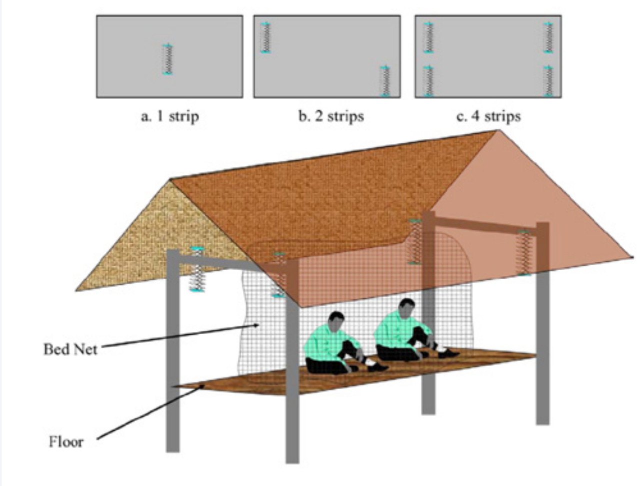


**Figure 3** Field test scene showing the metofluthrin strips in a beruga where Lombok people spend every evening prior to going to bed. Two treatment regimes were rotated on a daily basis (4 replications for the four different beruga) among the beruga; a. a. 4-plastic strips, b. 2-plastic strips and c. untreated control for the plastic strip trial, respectively. Two humans (males aged 20 to 30, 50 to 60 kg weight) laid in a bed net (ca 2 by 2 by 2 m), which was hung in each beruga, during the test as human baits, and mosquitoes were collected outside the net with aspirator. Strips were hung below the ceiling of the beruga outside the bed net.

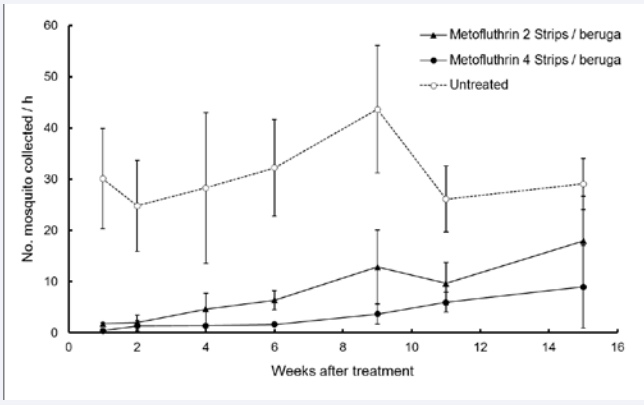


**Figure 4** Changes in the total number of mosquitoes (*Anopheles sundaicus, An. balabacensis, and Culexquinquefasciatus*) collected per hour during the trial of metofluthrin-impregnated plastic strips on Lombok Island, Indonesia.

rate of metofluthrin to approximately 50% of that of the previous plastic device. The new prototypes of metofluthrin-impregnated plastic strips were evaluated against malaria vector, *An. gambiae* Giles complex, in the Kongo villages of Bagamoyo district in coastal Tanzania [32]. The study used 20 houses (10 intervention and 10 control) and was conducted over 124 days. After the intervention with metofluthrin-impregnated strips (5–124 d), the mosquito density indices of the intervention houses were significantly lower than those of the control houses when the collection was performed by using the pyrethrum spray sheet ($F = 4.61$, 1 df, $P = 0.038$; 98.7% reduction in the total mosquito collection when compared with that for the controls) (Table 1).

Recently, Kawada et al., (unpublished data) found metofluthrin-impregnated plastic strips to be significantly effective at reducing invasions of a pyrethroid-resistant *An. gambiae* s.s. Population, which has developed > 90% homozygous L1014S point mutation at the voltage-gated sodium channel (*kdr*) in western Kenya. The number of mosquitoes was significantly lower than control houses in the houses with two metofluthrin-impregnated strips (10% w/w in 5g plastic net) / 10 m$^2$ (Figure 5).

Table 2 lists the environmental factors and the effective duration of the metofluthrin-impregnated plastic strips for the intervention houses in Tanzania [32] and in My Tho city, Tien Giang, Vietnam, where a similar metofluthrin trial was conducted in the same season in 2005 [33]. Variables including average temperature and humidity were calculated on an hourly basis from June 20 to August 3, 2006 for Bagamoyo and from June 20 to September 4, 2005 for My Tho. The room temperature was lower and the humidity was higher in Bagamoyo houses compared to

**Table 1:** Changes in the *Anopheles gambiae* s.l mosquito density indexafter intervention of metofluthrin-impregnated plastic strips in Bagamoyo, Tanzania.

| Days after Interven-tion | Mosquito Density Index (No. of female mosquitoes/ house/day) | | | |
|---|---|---|---|---|
| | Intervention (95% CI) | | Control (95% CI) | |
| 20 | 0.2 | (0.4) | 2.0 | (1.0) |
| 34 | 0.2 | (0.4) | 11.4 | (5.6) |
| 61 | 0.0 | (-) | 8.0 | (4.2) |
| 89 | 0.0 | (-) | 7.2 | (4.4) |
| 124 | 0.0 | (-) | 2.4 | (2.3) |

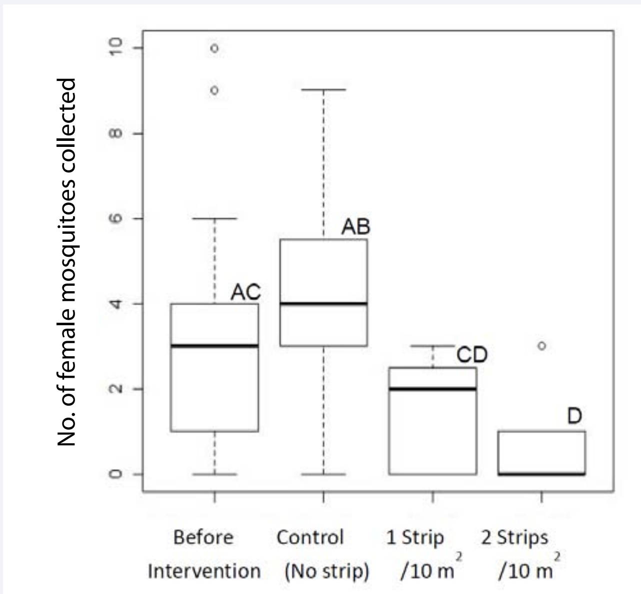


**Figure 5** The number of female *Anopheles gambiae s.s.* (pyrethroid-resistant wild population that has developed > 90% homozygous L1014S point mutation in the voltage-gated sodium channel) collected in the metofluthrin treated houses (1 strip/10 m$^2$ and 2 strips/10 m$^2$ at 1 week after intervention) in western Kenya. The different letters indicate significant differences between the Generalized Linear Mixed Model interventions.

**Table 2:** Environmental factors and properties of the metofluthrin-impregnated plastic strips within intervention houses for two trial sites and their respective target mosquitoes.

| Environmental factorsand strip properties | My Tho (2005)[2] *Aedes aegypti* | | Bagamoyo (2006)[2] *Anopheles gambiae* s.l. | |
|---|---|---|---|---|
| Average temperature (˚C)[1] | 29.1 | (0.8) | 24.8 | (0.7) |
| Average humidity (% RH)[1] | 70.1 | (5.1) | 75.3 | (3.9) |
| Total floor area ($m^2$/house) | 32.1 | (10.5) | 22.0 | (14.1) |
| Total volume ($m^3$/house) | 129.3 | (59.4) | 58.7 | (45.7) |
| Total opening area ($m^2$/house) | 6.6 | (5.0) | 5.7 | (4.3) |
| Corrected Opening Area/Volume | 0.051 | | 0.098 | |
| No. of metofluthrin strips/$m^2$ | 0.31 | | 0.52 | |
| Amount of metofluthrin (mg/$m^2$) | 191 | | 320 | |
| Effective Duration of metofluthrin strips (weeks) | 8 | | > 18 | |

[1]June 20 - August 3, 2006 in Bagamoyo; June 20 - September 4, 2005 in My Tho

[2]Figures in parenthesis are standard deviations

the corresponding conditions in the My Tho houses. Although the floor area and the structure volume were larger in My Tho houses, compared to those in Bagamoyo houses, the corrected opening area per total average volume of the houses in Bagamoyo was almost twice that of houses in My Tho, thereby indicating that the Bagamoyo houses are more "open" than the My Tho houses [32].

Metofluthrin-impregnated strips significantly reduced the density index of mosquitoes under several environmental conditions within the intervention houses in Vietnam (*Aedes aegypti*), Indonesia, Tanzania, and Kenya (malaria vectors and *Culex* species). Kawada et al., reported that mosquitoes were repelled by airborne metofluthrin vapors through the two main modes of pyrethroid action, i.e., knockdown activity and biting inhibition or disruption of orientation toward the host [29-33]. Of these, the latter may be categorized as a sub-lethal effect that results from neural excitement, which appears to occur at an earlier stage of pyrethroid toxicity [34-36]. Kawada et al., reported that both an increase in the average room temperature and a decrease in the open area of the rooms treated with metofluthrin-impregnated strips had an increased spatial repellent effect [33]. Paradoxically, in our recent field test in Malawi, we found that the evaporation rate of metofluthrin was not positively related to room temperature (Kawada et al., unpublished data). The above facts also suggest that the evaporation rate of metofluthrin was much higher in the well-ventilated houses, such as thatched-roofed houses with large open eaves, than in houses with small eave openings, thereby indicating the importance of air movement for removing and accelerating the release of the active ingredient (AI) from the surface of the strips. The corrected opening area/volume ratio in the Bagamoyo houses was nearly twice as high as that of the houses in My Tho city (Table 2). The large and numerous open eaves in typical rural African houses are considered to be important nighttime entrances for *An. gambiae* [12,37,38]. However, these openings might increase the evaporation rate of metofluthrin and result in high mosquito control efficacy. Therefore, we cannot simply conclude that the large opening area of the Bagamoyo houses negatively affects the spatial repellent efficacy of metofluthrin.

## House screening with Olyset® Nets in a Kenyan malaria endemic area

There have been several attempts to use LLINs to control other vectors, including *Ae. aegypti* [39-43] and *Phlebotomus* [44-48]. Olyset® Net and/or PermaNet® LLINs have also been used as curtains [39,40,42,49,50] and water container covers [42,43,49,50]. Eaves are thought to be the most important entrance for malaria vectors. The use of nets to screen ceilings and eaves is likely to provide the greatest benefit in moderating disease transmission [12,51] and will be more readily accepted if the nets have a coarse mesh size to provide optimum ventilation.

Small-scale trials using Olyset® Net materials were performed in Mbita, Nyanza province, western Kenya in 2010 and 2011. *An. gambiae* s.s., *An. arabiensis*, and *An. funestus* s.s. are the main malaria vectors in this area and were recently reported to have developed multimodal pyrethroid resistance [52]. *Anopheles rivulorum*, which is a sibling species in the *An. funestus* complex, is also a minor vector in this area [53]. Olyset® Nets impregnated with 2% permethrin were used in the study. The net materials were cut and sewed into 7 × 5 m sheets and ring bands were attached along the diagonal of the nets for hanging under the ceiling (Figure 6). The study was performed in three houses (two interventions, one control) in Nyandago village in Gembe East, Mbita Division in the Suba district of the Nyanza province, western Kenya. The shielding effect of the ceiling nets was evaluated by counting the number of indoor resting mosquitoes collected using a battery-powered aspirator. The Olyset® net covering the ceiling and the eaves resulted in a significant reduction in the number of resting mosquitoes (a mixed population of *An. funestus* s.s. and *An. arabiensis*) inside houses [54]. In the intervention houses, the number of mosquitoes drastically decreased 1 day after the installation of ceiling nets and lower densities were maintained for the 9 months until the removal of the nets; the mosquito density in the control house remained at a high level during the experimental period (Figure 7). Lindsay et al. reported that insecticide-treated nets provided marginal protection compared to that of untreated screen nets [12]. The present study, however, emphasizes the necessity of using pyrethroid-impregnated nets as a chemical barrier, although this protection may be partly due to the increased ventilation from the coarse mesh size of Olyset®

Nets (Figure 8).

A large-scale field trial of ceiling nets combined with LLINs (Olyset® Net) in 1800 houses in western Kenya was started in 2001. A significant reduction in the number of mosquitoes (mixed population of *An. funestus* s.s. and *An. arabiensis*) and *P. falciparum* positive cases in the intervention houses was observed for over 12 months after initial intervention (Minakawa et al., unpublished data). Screening ceilings and closing eaves with insecticide-treated nets with a coarse mesh size, such as the Olyset® Net, will be accepted by residents, and is a cost-effective and environmentally safe way to prevent mosquitoes from entering houses.

## DISCUSSION AND CONCLUSION

For many decades, pyrethroids belonging to the knockdown agent group have been globally successful as spatial repellents. Spatial repellency will not induce pyrethroid resistance because it results in low fatality rates and selection pressure on the affected insect populations. The discovery of the phenoxy benzyl alcohol moiety accelerated the development of photostable pyrethroids for outdoor use, including their use in agriculture. These "second-generation" pyrethroids have been used worldwide as effective vector control agents using various application techniques, such as residual spraying, ULV (ultra-low volume) spraying, and LLINs. However, photostable and highly effective pyrethroids have accelerated the development of pyrethroid resistance in mosquito populations. Photostable pyrethroids consist of two structurally different types of chemicals depending on the presence of either α-cyano moiety, type I (permethrin, bifenthrin, etofenprox, etc.) or type II (deltamethrin, λ-cyhalothrin, cypermethrin, etc). Olyset® Net is a slow-releasing device composed of plastic fibers impregnated with permethrin—one of the most popular and safe type I pyrethroids. Siegert et al. reported that the Olyset® Net reduced mosquito landing attempts and elevated their flight frequency, resulting in low mortality [55]. However, mosquito landing attempts on the PermaNet®, containing a type II pyrethroid (deltamethrin) and under the same conditions, were more sustained and caused greater mortality than those on the Olyset® Net. This appears to be important for the effective control of the mosquito population. Highly lethal pyrethroids with less excito-repellency appear to be the most effective at reducing vector mosquito populations, although such highly lethal pyrethroids might accelerate the development of pyrethroid-resistance. However, the excito-repellency of slow-released

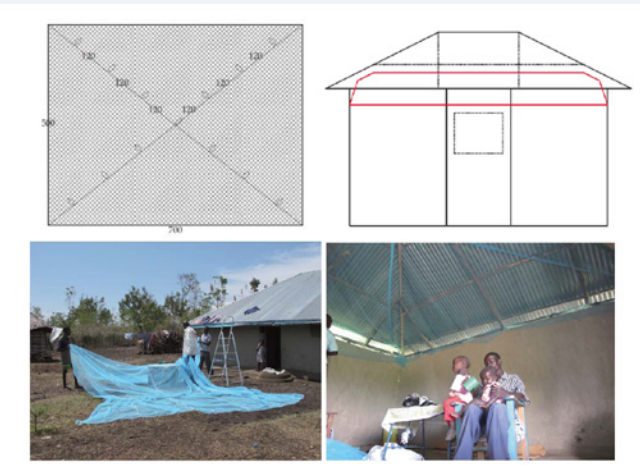

**Figure 6** Ceiling net using Olyset® Net materials (upper left) and an outline sketch of the ceiling net installed in a house (upper right). Intervention scene with a ceiling net (bottom left & right).The study was performed in 3 houses in Nyandago village, Gembe East, Mbita Division, Suba district of Nyanza province, western Kenya. Two houses (NYAND 8 and 11) were used as intervention houses, while the third (NYAND 6) constituted a control. One-and-a-half ceiling nets (7 × 5 m plus 3.5 × 5 m) were installed to cover each house. The bottom edges of the ceiling nets were stapled onto the edge of eaves or mud walls, to close the openings of the eaves.

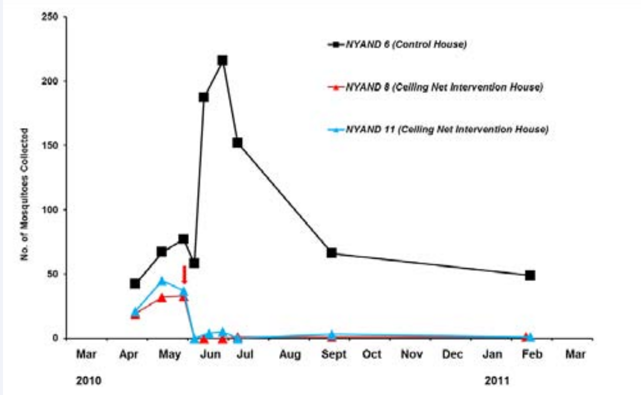


**Figure 7** Differences between the numbers of mosquitoes (mixed population of *Anopheles funestus s.s. and An. arabiensis*) collected in the ceiling net intervention houses (NYAND 8, 11) and those collected in the control house (NYAND 6). The red arrow indicates the day of intervention.

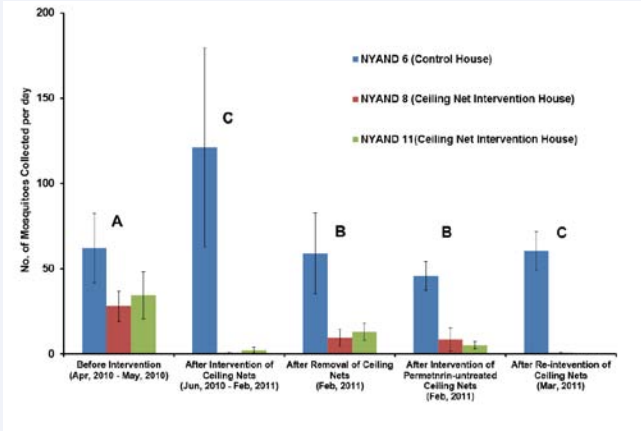


**Figure 8** The average number of mosquitoes collected before intervention of permethrin-impregnated ceiling nets, after intervention, after removal of the permethrin-impregnated ceiling nets, after intervention of permethrin-untreated ceiling nets, and after re-intervention with new permethrin-impregnated ceiling nets. Bars indicate 95% confidence limits. The same letters indicate no significant difference from Tukey's HSD test (P = 0.05) of the square root of the ratio of the number of mosquitoes collected in the intervention house to the number collected in the control house, converted into Arcsin.

permethrin might reduce human-vector contact and blood feeding success. For example, there was no difference between the field efficacies of Olyset® Net and PermaNet® as measured by blood feeding rate [56]. The positive use of excito-repellency of slow-released pyrethroids, therefore, might lead to bio-rational vector control with the maximum reduction of mosquito biting and the minimum risk of resistance. Little is known about the relationship between the mode of insecticide resistance and excito-repellency in pyrethroid-resistant mosquitoes. Recently, Kawada et al. reported a different repellent reaction in field-collected *An. gambiae* s.s., *An. arabiensis*, and *An. funestus* s.s. From western Kenya [57]. Pyrethroid-resistant *An. gambiae* s.s. Populations governed by knockdown resistance (*kdr*), were found to lose repellency to pyrethroids, whereas the other mosquito populations lacking *kdr* (*An. arabiensis*, and *An. funestus* s.s.) maintained high repellency irrespective of their possession of metabolic resistance to pyrethroids [57] (Figure 9). The above finding might inform the development of new personal protection measures using the excito-repellency of pyrethroids, but additional genetic evaluation should be conducted to further support this avenue for research.

Humans have invented insecticides to ensure comfort and achieve ideal conditions. Insecticides should be as effective as possible in order to realize these goals, but development and manufacturing costs should be kept to a minimum [58]. It is, therefore, our duty to use insecticides in the most effective and prudent manner possible in order to maintain their effectiveness and longevity. In order to effectively manage pyrethroid resistance, the establishment of a feasible insecticide management system and a regular monitoring system of pyrethroid susceptibility is essential. Moreover, it is expected that, in the future, new protection measures using excito-repellent type I pyrethroids will be of great interest either as substitutes or as supplements for bio-rational vector control measures. In addition, the use of photo-unstable knockdown agents as spatial repellents will likely increase because they effectively interfere with disease transmission without causing selection pressure to insect populations.

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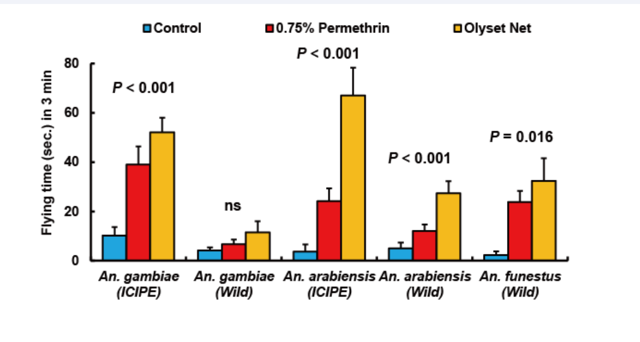


**Figure 9** Cumulative flying time of female *Anopheles gambiae s.s.*, *An. arabiensis*, and An. funestus s.s. during a 3-min exposure to an Olyset® Net, 0.75% permethrin-impregnated paper, or untreated net material, using a modified WHO cone bioassay. Letters indicate the significance levels form a Kruskal-Wallis test; bars indicate the SEs.

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