Insecticide-free trapping bed-net can mitigate insecticide resistance threat in malaria vector control strategies

Chouaibou S. Mouhamadou*[1,2], Kun Luan*[3], Behi Kouadio Fodjo[1], Andre West[4], Marian McCord[3], Charles S. Apperson[2,5], R. Michael Roe*[2]

[1]*Centre Suisse de Recherches Scientifiques en Côte d'Ivoire* (CSRS), Abidjan, Côte d'Ivoire

[2] Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC

[3]Department of Forest Biomaterials, North Carolina State University, Raleigh, NC

[4]Department of Textile and Apparel, Technology and Management, North Carolina State University, Raleigh, NC

[5]Comparative Medicine Institute, North Carolina State University, Raleigh, NC

cmouham@ncsu.edu

kluan@ncsu.edu

ajwest2@ncsu.edu

behikouadio@gmail.com

mmccord@ncsu.edu

apperson@ncsu.edu

mroe@ncsu.edu

*corresponding authors

Abstract

Mosquito-borne malaria kills 429,000 people each year with the problem acute in sub-Saharan Africa. The successes gained with long-lasting pyrethroid treated bed-nets is now in jeopardy because of wide-spread, pyrethroid-resistance in mosquitoes. Using crowd modeling theory normalized for standard bed-net architecture, we were able to design a pull-trap-kill mechanism for mosquitoes that does not require insecticides. Using three-dimensional polyster knitting technology and heat fixation, trap funnels were developed with high capture efficacy, no egression under laboratory conditions, and greater durability than bed-nets now on the market. In field studies in Africa in WHO huts, we found the Gen3 T (trap)-Net with these cones had a 4.3-fold greater trap-kill rate with no deterrence or repellency effects compared to Permanet 2.0, the most common bed-net deployed presently in Africa. A T-Net population model was developed based on field data to predict community level mosquito control compared to a pyrethroid bed-net. This model showed the Gen3 T-Net under field conditions in Africa against pyrethroid resistant mosquitoes was 12.7-fold more efficacious than single chemical pyrethroid treated nets, demonstrating significantly greater mosquito control using bed-nets without insecticides.

Introduction

Malaria is the leading cause of morbidity and mortality in Sub-Saharan Africa with 212 million cases estimated by the WHO and about 429,000 deaths each year[1]. Malaria prevention is mainly based on vector (mosquito) control, using insecticides either as indoor residual sprayings (IRS) or in long-lasting insecticide-impregnated mosquito bed-nets (LLINs)[2]. The efficacy of these measures depends primarily on the susceptibility of the mosquito to insecticides. An estimated 663 million cases of malaria have been averted in sub-Saharan Africa since 2001 as a result of the scale-up of malaria control interventions; 69% of these cases were a direct result of the use of LLINs[3]. Unfortunately, mosquitoes have become resistant to insecticides in recent years[4-6] (including pyrethroids, the only class of insecticides presently used in bed-nets), threatening the efficacy of insecticide-based vector control programs[7-9]. This situation is problematic and highlights the urgent need to develop new strategies to mitigate development and spread of insecticide resistance and preserve the efficacy of currently available insecticides and malaria control interventions.

Development of a new, bed-net insecticide chemistry that is safe to human exposure every sleep cycle, that must survive for years of human handling and washing, and will not promote mosquito resistance and cross-resistance is not impossible but a major challenge[10]. Alternative solutions include the reformulation of agricultural chemicals for vector control with completely different modes of action from the common public health

insecticides. However, it is difficult with these solutions to account for prior and future exposure of mosquito larvae to pesticides used in farming resulting again in resistance[11]. All of these challenges have led us to focus on a different approach, a mechanical killing solution for mosquito control.

It is well known that mosquitoes are attracted to carbon dioxide and other human odors, and these odorants emitted from the body are warmer than ambient air and rise from its source[12]. Infrared video tracking has now shown that about 75% of mosquitoes follow this odorant plume and are attracted to the top surface of a bed-net in use[13,14]. Hence, we hypothesized that mosquito movement in the absence of visual and odorant cues would be random. If we then treat mosquitoes as ideal particles in an ideal gas, we can use the Maxwell-Boltzmann distribution[15] to develop a mathematic model for the construction of an insecticide-free trapping bed-net (T-Net) to capture and hold host-seeking mosquitoes during and after a sleep cycle. The T-Net is a bed-net with two compartments, including (i) a lower sleeping compartment and (ii) an upper mosquito trap containing cone-like funnels as mosquito entry points into the trap space. The 3-D knitting technology was used for cone construction. Proof of concept was investigated in the laboratory and then later under field conditions with wild resistant *An. gambiae* mosquitoes in Tiassale, Côte d'Ivoire (Africa) to verify our model. From the field data, we developed a second model to describe community level mosquito control by the T-Net versus the most common bed-net deployed in Africa.

Results

Crowd model applied to trap compartment

A T-Net model was developed based on the volumetric parameters of a bed-net and the flying behavior of mosquitoes. In this model, each mosquito is treated as an ideal gas particle, and the flight track assumed to be random. In addition, the Maxwell-Boltzmann distribution[15] was used for counting the random flight of mosquitoes with different velocities. The trap model is based on the architecture of the WHO approved experimental research huts[16] in the region of Tiassale in Cote d'Ivoire (Africa), where our field trial was conducted (Fig. 1a). Figure 1b diagrams the process by which mosquitoes can fly from an open area in the hut and into the T-Net cones and trap space. It can be assumed that (i) the flight track of mosquitoes is random (Fig. 1c); (ii) the diameter of the hole is small enough that will not affect the flight path of other mosquitoes; and (iii) the trapping does not perturb the flight velocity of the remaining mosquitoes in the hut. Figure 1d assumes a hole area dA, a mosquito that is at distance vdt from the hole and a mosquito moving at a speed v at an angle θ from the normal surface toward the area dA. All mosquitoes within a parallelepiped volume around the dA area moving toward the hole with speed v will pass through the top opening of the

cone in the time interval dt. Therefore, the total number of mosquitoes flying through the dA area in the time interval dt is

$$N_A = \rho v cos\theta dA dt \tag{1}$$

where ρ is the mosquito density in the hut.

Assuming the distribution of individual mosquito velocities obeys the Maxwell-Boltzmann distribution, which was first defined and used for describing particle speeds in idealized gases, one integrated expression of the distribution relates particle density with average velocity. Similarly, the average trapped mosquito number per area per time is:

$$\dot{N} = C\bar{v}\rho \tag{2}$$

where $\bar{v}$ is the average fly speed, ρ is the mosquito density in the hut, C is a constant determined by the active behavior of the mosquito (1/4 in ideal gas theory).

As mentioned above, most of mosquitoes fly on the top of bed-net and thus the work volume is

$$V_w = V - V_{net} - V_s \tag{3}$$

where V is the hut volume, $V_{net}$is the bed-net volume and V_s is the volume surrounding the bed-net except the volume on the top of bed-net.

Assuming mosquitoes are distributed evenly on the top of the bed-net, the trapped mosquito number for the T-Net in testing time $t\ is$:

$$N = C \times a \times \frac{N_{hut} \times \bar{v}}{(V - V_{net} - V_s)} \times t \tag{4}$$

where N_{hut} is the total mosquito number in the hut, C is a constant and a is the bottom area of the cone.

Thus, the T-Net model relates trapping number with container volume and flight velocity. If the number of mosquitoes in a hut is a variable, and other parameters are constant, the predicted results of equation (4) are shown in Figure 1e; if V_{hut} is a variable, the predicted trapping number for each generation T-Net is shown in Figure 1e.

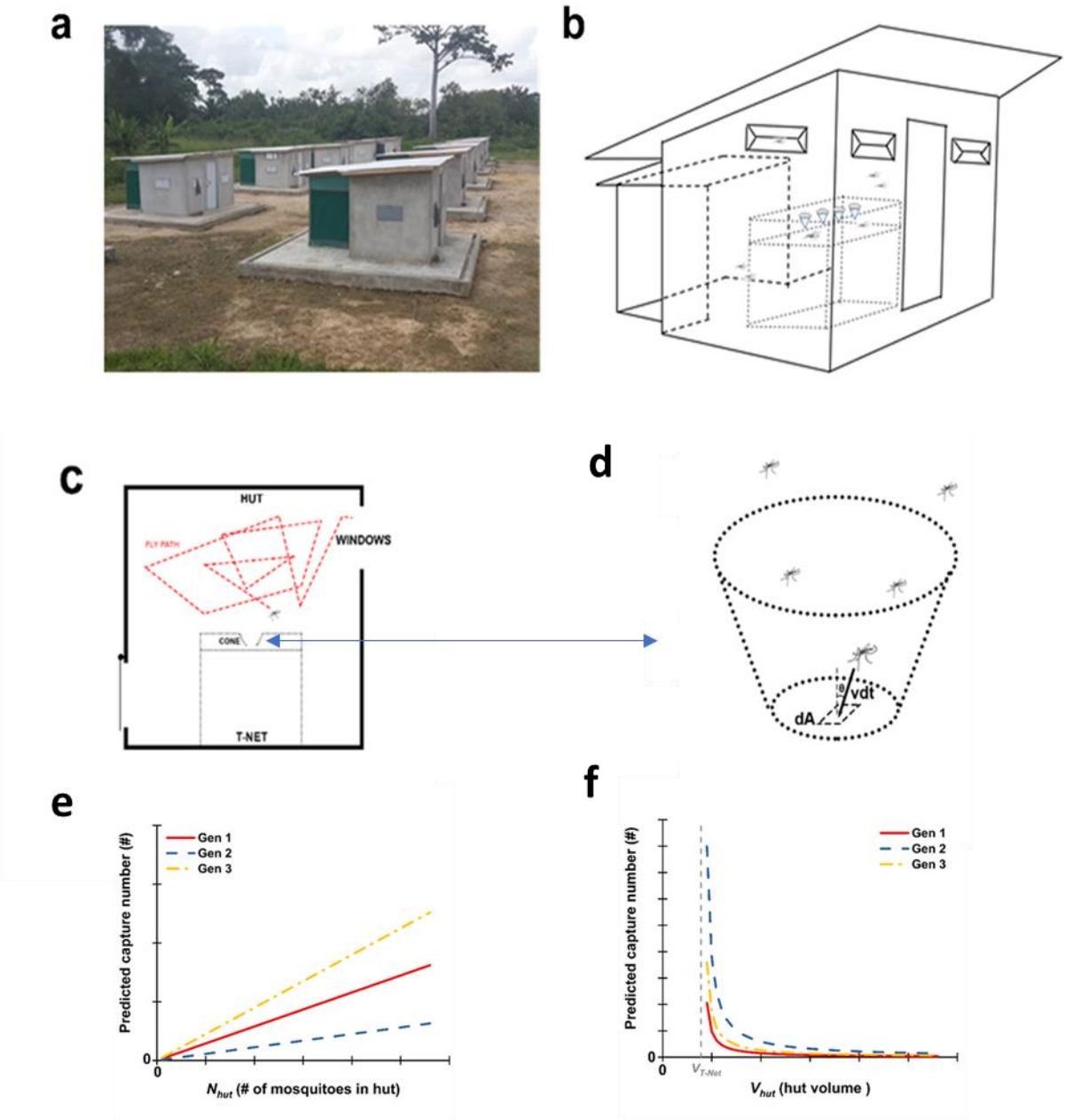

Figure 1. Principle and model of the T-Net: (a): picture of experimental huts in the region of Tiassale in Cote d'Ivoire (Africa), where the field trial was undertaken; (b): schematic illustration of hut. (c): schematic illustration of mosquito flight track in the hut; (c): unit area on the bottom of T-Net that captures mosquitoes, (e): predicted capture number for Gen 1, Gen 2 and Gen 3 T-Nets when uncertain number of mosquito flying into hut, (f) predicted capture number for Gen 1, Gen 2 and Gen 3 T-Nets when hut volume increases and the number of mosquito flying into hut is constant for each generation T-Net.

Model prediction and T-Net confection

The attraction of mosquitoes through the funnels by human odorants is hypothesized to be mostly caused by carbon dioxide from human respiration. Therefore, it was possible that funnels were needed only at the head of the sleeping area. From this assumption, we designed and constructed our first generation (Gen1) of T-Net. It was made of a circular aggregate of 7 cones of 10 cm of large diameter, 8 cm deep and 1.8 cm of small diameter sewn at the central-head part of the bed-net roof at 30 cm from the edge, and opened on a trapping box of 25 cm deep (Fig. 2a). With this Gen1 T-Net, our model showed a fairly accurate prediction of trapping rate as shown by the regression curves (Fig. 1e&f). However, we wanted to increase the trapping rate. So, we designed the Gen2 T-Net. It was made with one large cone of 15 cm of large diameter, 12 cm deep and 3 cm small diameter fitted on a cylindrical trapping bag of 25 cm deep and 25 cm diameter fixed at the central-head part of the bed-net roof at 30 cm from the edge (Fig. 2b); From the equation (4), we made a catch rate prediction with Gen2 T-Net. We obtained a regression curve which stipulated a lower catch rate (Fig. 1e&f) than that observed with Gen1 T-Net.

On one hand, by further increasing the size of the cones, we could at the same time facilitate the escape of mosquitoes after they have been trapped. On the other hand, having up to seven cones per net could be more complicated to make and more expensive. On Gen1 and Gen2 T-Nets, the idea was to fix the cones above the sleeper's head; but what if the other parts of the sleeper's body could also positively affect the capture rate? To verify this hypothesis, we decided to conduct a laboratory experiment where the passage rate of mosquitoes through the cone of Gen2 would be assessed.

Knitting of cone and assessment of mosquito passage

- Knitting of cones

Initially, we considered using plastic cones. But given the difficulty of fixing them on the bed-net fabric and their non-permeability to carbon dioxide, we opted for knitted cones. Knitting of cones was completed on a flat computerized knitting machine, SWGN2 (Shima Seiki, Japan). This gauge was selected so that the resulting cone fabric would have openings between loops similar to those of traditional bed-nets. Similar to the construction of stockings or socks, cones were knitted in-the-round to make a 3D shape (Fig. 2d). Use of this construction method allowed us to alter the shape, depth, width, and height in all sections of the cone to meet our design demands. The knitted cones had three main parts: a large opening at the mouth (which was designed to allow for passage of mosquitoes into the cone), the tapered cone walls (which were designed to direct the movement of mosquitoes into the trapping compartment), and the neck of the cone opening into the trapping compartment (the size of the neck was optimized to

allow passage of mosquitoes yet abate the egression of trapped mosquitoes). To prevent the collapsing and flattening of the knitted cones, they were constructed with thermal bond fibers. This yarn is a polyester binder spun yarn in which each fiber is composed of a sheath part (co-polyester) and a core (regular polyester). The bursting strength and durability were further assessed in comparison to conventional bed-net material (Extended data).

- Tunnel test for cone efficiency

Effectiveness of cones in allowing mosquito passage was successfully evaluated in modified WHO tunnel tests[17]. Figure 2e shows the designed experiment. The results of two replicates of 50 and 106 mosquitoes revealed a mean passage/trapping rate of 49.50% and 0% of egression/escape after 2 hours in the absence of bait (Fig. 2f).

From these findings, we supposed that an arrangement of cones along the trap compartment roof could increase the rate of mosquito trapping. Hence the Gen3 T-Net was built. It was fitted with four large cones (less in number than Gen1) of 15 cm of large diameter, 12 cm deep and 3 cm small diameter (same as Gen2), aligned lengthwise along the center line of the bed-net with the cones spaced 26 cm apart. All cones opened on a trapping box of 25 cm deep (Fig. 2c); our model predicted an increase in the trapping rate with Gen3 compared to Gen1 and Gen2 as shown by the regression curves (Fig. 1e&f). Proof of concept study to test the trapping efficiency of Gen3 T-Net was carried out in laboratory conditions with the susceptible laboratory colony of *An. gambiae* Kisumu. Mosquitoes were freed in a walk-in cage containing a T-Net with someone sleeping underneath.

A total of 300 female mosquitoes were used in six replicates of an average of 50 mosquitoes per replicate (range = 41-85 females). The trapping rate ranged from 45.28% to 86.90% with an average rate of 67.7% (± 20.5% CI). No mosquito egression from the trapping compartment was observed at any time point (30 minutes, 1h, 2h, 6h and overnight) for any of the trial replicates (Fig. 2f).

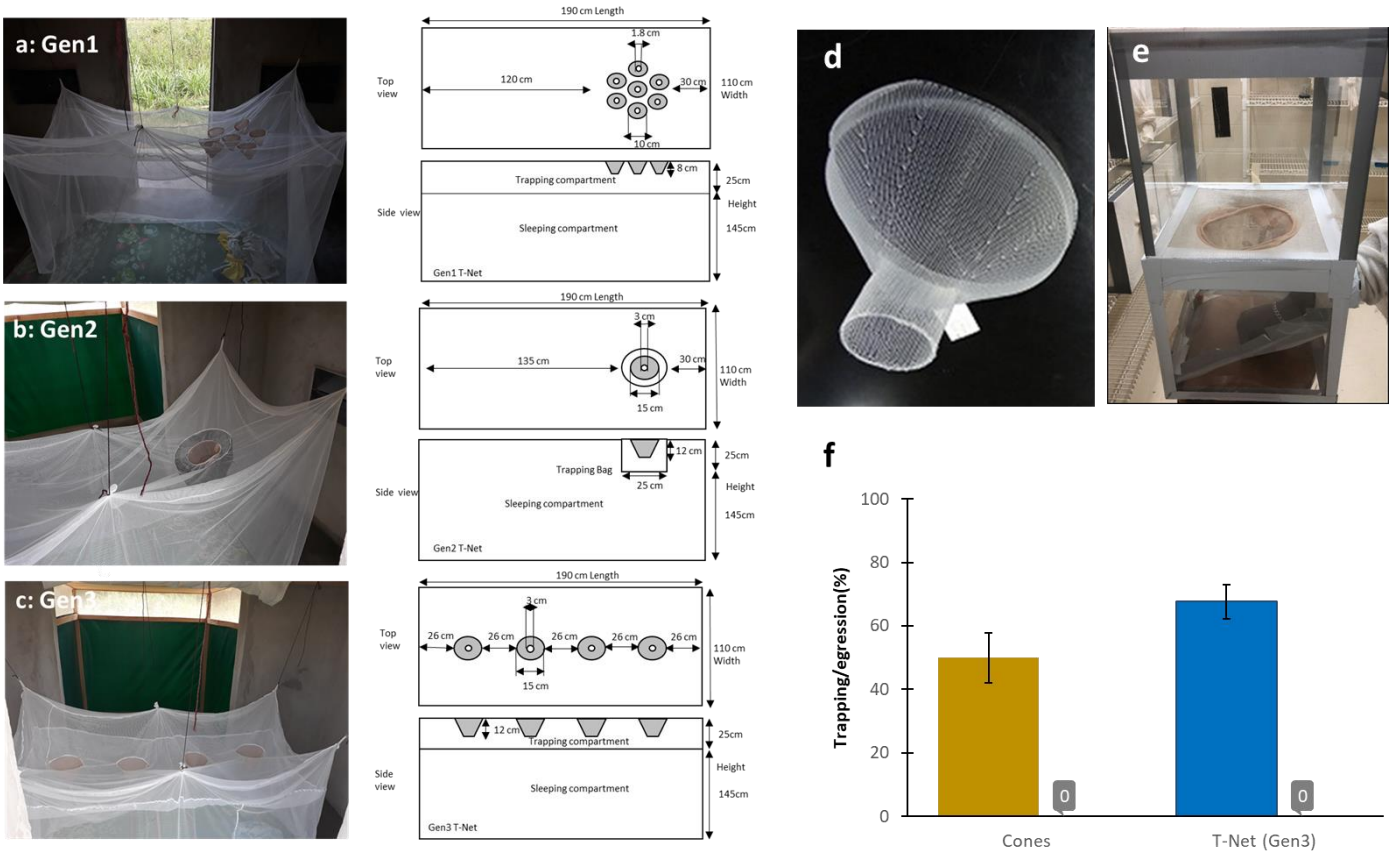

Figure 2: Different designs of T-Nets and proof of concept findings. (a): Gen1 T-Net made of a circular aggregate of seven small cones; (b): Gen2 T-Net made with one large cone fitted on a trapping bag; (c): Gen3 T-Net fitted with four large cones; (d): Knitted cone; (e): experimental tunnel apparatus to assess the trapping efficacy of knitted cones; (f) results obtained on tunnel test for the trapping efficiency of cones and with the Gen3 T-Net as trapping potential proof of concept. The histograms represent the trapping rates, the bars on top are the confidence intervals and the “0” are the exit rates or egression in absence of bait.

Field performance of Gen1, Gen2 and Gen3 T-Nets against insecticide resistant, wild free-flying mosquitoes

The performance of T-Nets was compared to that of Permanent 2.0 (PN2.0)-LLIN, the most used LLIN in Africa.

The field trial was carried out in WHO-approved experimental huts[16] in the locality of Tiassale where wild *An. gambiae* malaria vectors are resistant to insecticides[18,19]. Three entomological parameters were measured: (i) the mean entry rate of mosquitoes per hut per night, (ii) the mean exit rate per hut each night, and (iii) the mean mortality rate per hut per night which consisted of mosquitoes that were found trapped (alive or dead), and dead mosquitoes for the treated net.

- Mean entry number

A total of number of 53, 139, 175 and 156 *An. gambiae sl* mosquitoes were collected in 14 nights representing 14 observations, respectively, for PN2.0, Gen1, Gen2, Gen3 and (Table 1). The mean number of mosquitoes entering each hut per night was 3.8 (σ= 2.96) for PN 2.0 hut which was the lowest, followed by 9.9 (σ= 10.1) for Gen1, 12.5 (σ= 10.5) for Gen2 and 11.1 (σ= 8.3) for Gen3. The difference was not significant between Gen1, Gen2 and Gen3, but a significant difference was observed between PN 2.0 and each of the T-Nets ($P<0.05$) (Fig. 3a).

- Mean exit rate

As expected, the PN2.0 showed the highest mean exit rate of mosquitoes per night, which was 77.2% (σ = 29.4) and significantly different from that of Gen1, Gen2 and Gen3. In Gen1, Gen2, we recorded a mean mosquito exit rate per night of 47.3% (σ= 27.7), 45.7% (σ= 30.6), respectively, and in Gen3, it was 26.7% (σ= 20.7) and was the lowest. No significant difference was observed between the three T-Nets (Table 1; Fig. 3b).

- Mean mortality rate

In terms of mortality (Fig. 3c), the lowest mortality rate was achieved by PN2.0 and was 14.3% (σ= 17.9). It was significantly different from that of all the other nets. In Gen1 and Gen2, we observed mortality rates of39.1% (σ = 30.5) and 39.8% (σ = 26.0) ,respectively, with no statistical difference ($P> 0.05$). The highest mean mosquito mortality rates per hut per night were recorded for Gen3 and was 61.9.5% (σ= 22.1). The difference was significant between this rate and those of Gen1, Gen2 and PN.20 ($P <0.05$).

T-Net model verification

Base on the tunnel test in Figure 2e, C in equation (4) was calculated by the condition parameters. The cage dimension was 30cm length, 30cm width and 30cm height. Cone trap diameter was 3cm.Thus, C is 0.00783. According to this equation, the prediction of

trap number for Gen 1, Gen 2 and Gen 3 were respectively 2.87, 1.41 and 5.02 corresponding to trapping rates of 28.9%, 11.3% and 45.06% (Fig. 4d). The model and the field trial showed the same trend, although the predicted catch rates were lower for the model. This difference was expected as the model expresses values without considering the human attraction exerted on mosquitoes. With the Gen2, much more mosquitoes were trapped in the field than was predicted. This finding suggests that the position of cones above the sleeper head where emanation of carbon dioxide would be greatest was sufficient to achieve a high trapping rate regardless of the number of cones.

Table 1: Results from field evaluation of Gen1, Gen2 and Gen3 T-Nets. Total caught, mean entry, mean exit and mean mortality rates per hut per night of *Anopheles gambiae* mosquitoes after 14 observations during which the insecticide-free T-Nets were compared to Permanet 2.0 (PN2.0)-LLIN in Tiassale (Cote d'Ivoire) where mosquitoes are resistant to insecticides.

			Entry #		Exit rate (%)		Killing rate (%)	
Variable	# obs.	Total caught	Mean*	Std dev.	Mean*	Std dev.	Mean*	Std dev.
PN2.0	14	53	3.8a	3.0	77.2a	29.4	14.4a	18.0
Gen1	14	139	9.9b	10.2	47.3b	27.8	39.1b	30.6
Gen2	14	175	12.5b	10.6	45.7b	30.6	39.9b	26.1
Gen3	14	156	11.1b	8.3	26.8b	20.7	61.9c	22.1

*Values not sharing the same letters are statistically significants
#obs.: number of observation
Std dev.: standard deviation

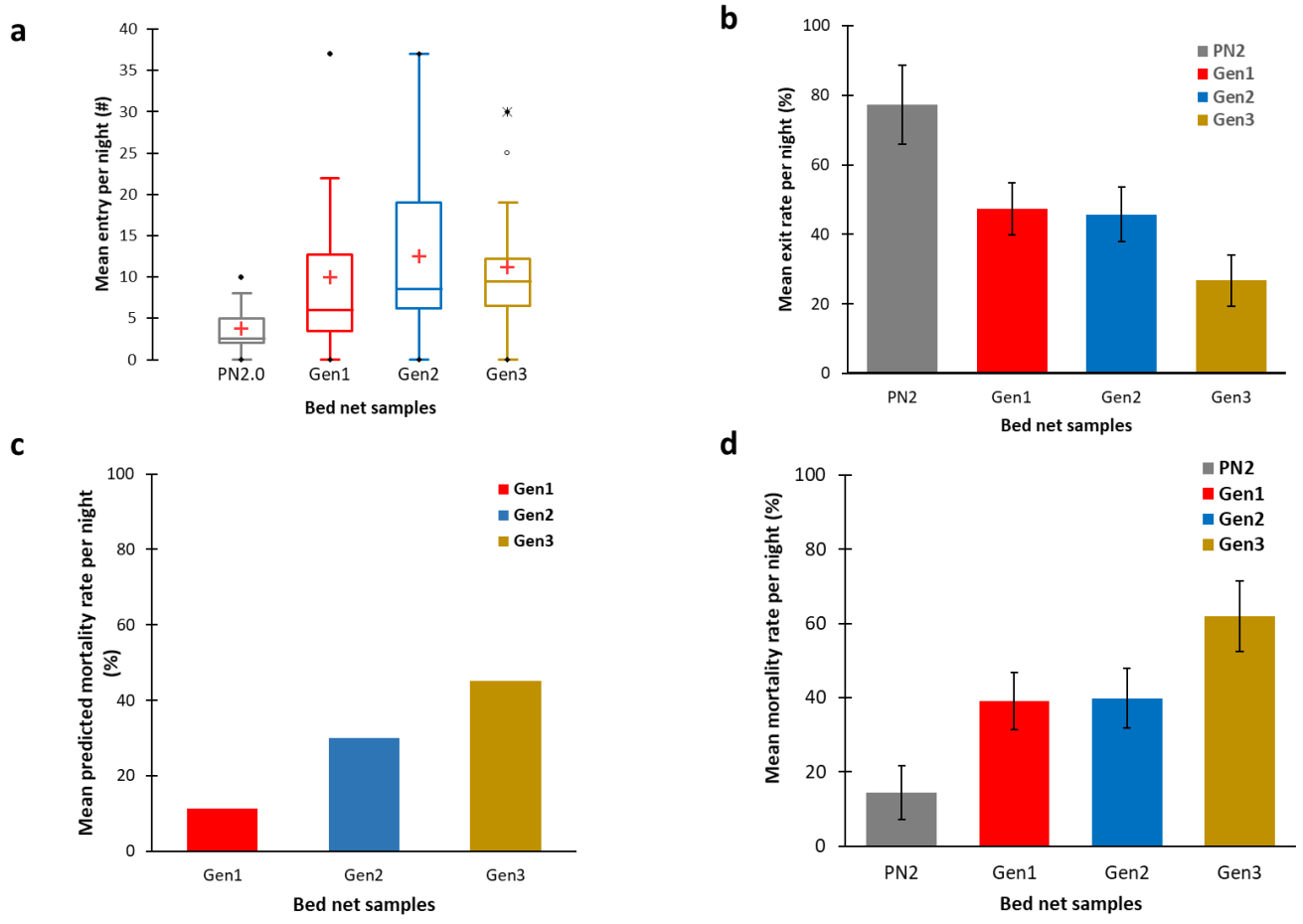

Figure 3: Performance of Gen1, Gen2 and Gen3 insecticide-free T-Nets in comparison to the Permanet 2.0 LLIN (PN2.0) LLIN in Tiassale (Cote d'Ivoire) where mosquitoes are resistant to insecticides. The nets were randomly allocated to four huts. Nets and sleepers rotated each night. Up to 14 observations were made. (a) shows the mean number of mosquitoes entering each hut per night. (b) is the mean exit rate per hut per night, (c) is the killing rate predicted by T-Net model; (d) is the mean mortality rate per hut per night including both trapped and dead mosquitoes. The bars on top of the histograms represent the confidence intervals.

Model of community level of mosquito control by T-Net

The actual fold change in killing rate between each of the T-Nets and PN2.0 calculated from field data were 2.7-fold for Gen1 and Gen2 and 4.3-fold for Gen3. When the deterrent effect exerted by insecticide on mosquitoes was considered, it was possible to estimate the overall relative mosquito control as follow:

$$Overall\ relative\ mosquito\ control\ = \frac{Mu}{(1 - \mathrm{D})\ x\ Mi}$$

Where:

- Mu is the mortality caused by the untreated T-Net (%);
- Mi is the mortality caused by the insecticide treated net PN2.0 LLIN (%);
- D is the dettrerent effect $\left(\mathrm{D} = \frac{(Eu - Ei)}{Eu}\right)$
- Eu is the mean entry number in untreated T-Net,
- Ei is the mean entry number in the insecticide treated PN2.0 LLIN.

The Gen3 outperformed Gen1 and Gen2; It was 12.7-fold more efficacious than PN2.0, followed by Gen2 (9.1-fold) and then Gen1 (7.1-fold) (Fig. 4).

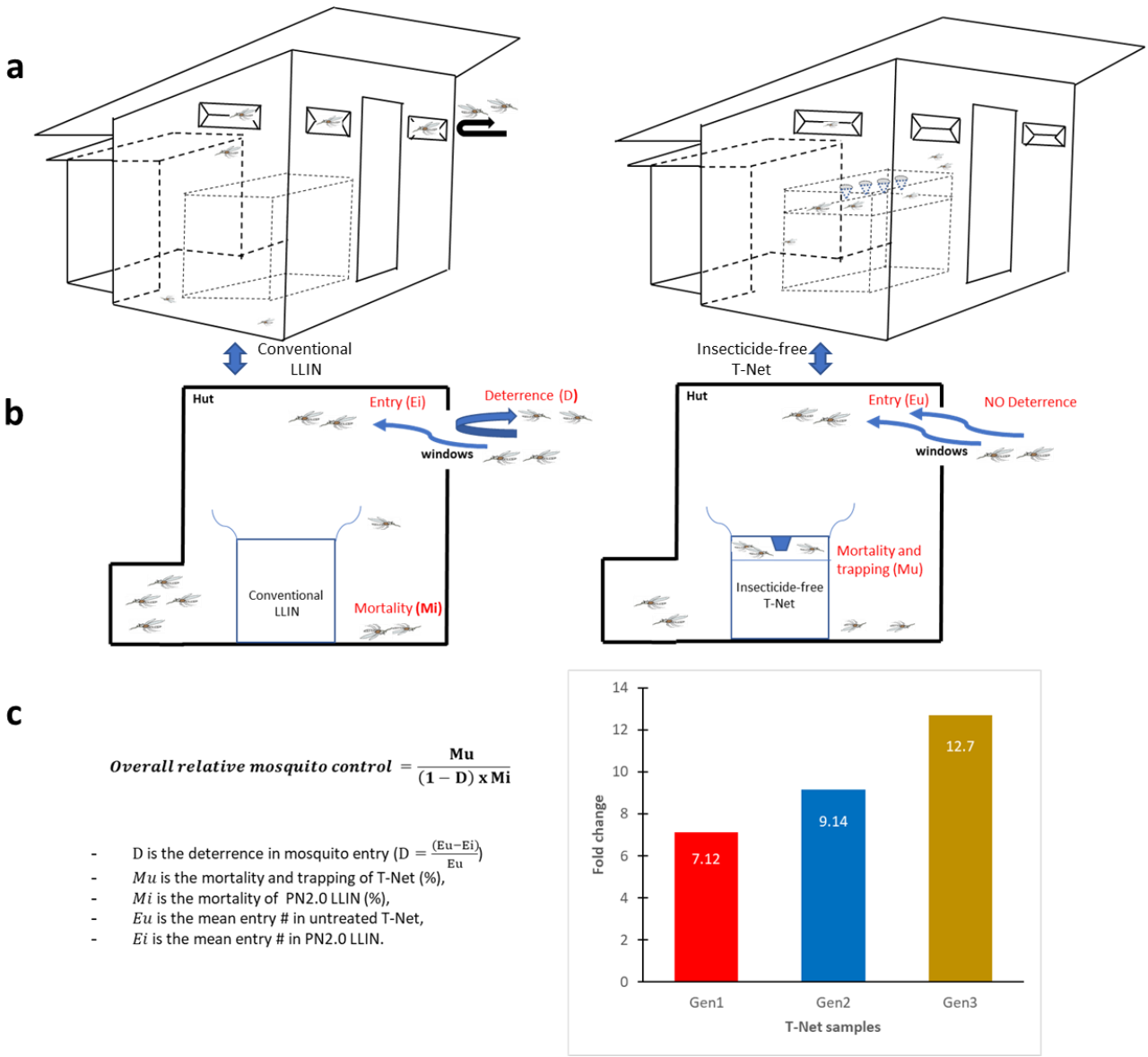

Figure 4: Overall relative mosquito control. (a): schematic illustration of the experimental hut with either a LLIN of a free-insecticide T-net; (b) highlights the different parameter used to define the model; (c) is the overall relative mosquito control. The histograms showed the increased mortality of mosquitoes over PN2.0-LLIN.

Discussion and conclusion

Malaria is a devastating disease, and vector control is a crucial factor in saving lives. However, the situation has become critical because mosquitoes have become resistant to core intervention tools such as LLINs or IRS. Tackling insecticide resistance by developing new approaches for vector control is imperative to control malaria.

The current model-driven insecticide-free trapping bed-net is a paradigm shift in malaria vector control. Its functionality is based on the attraction and trapping of mosquitoes regardless their insecticide resistance status. This study describes for the first time the efficacy of a trapping bed-net as a malaria vector control strategy, which addresses the problem of insecticide resistance. The advantage of the model is that it can predict the trapping rate and aid in the design of the T-Net accordingly.

As commonly found in experimental hut trials in which non-insecticide and insecticide-treated products are compared each-other [20-21], we noted higher mosquito entry rates in huts with T-Nets than that observed with PN2.0-LLIN. Similarly, the exit rate was higher for PN2.0-LLIN than that of the T-Nets. This is likely due to the excito-repellency property of insecticides. The excito-repellency effect can be perceived as beneficial to the extent that it keeps some mosquitoes away from the sleeper, and thus provides personal protection to the net user while the community protection is achieved by the insecticidal effect of the bed-nets when distributed at high coverage. But in the same time at the community level, the benefit of the excito-repellency effect is likely to be nullified and can even contributes to favor behavioral resistance and facilitates outdoor malaria transmission as mosquitoes are repelled outdoors and towards unprotected people [22,23]. Direct observation of field results showed a 2.7 to 4.3-fold increase mortality for the T-Nets comparing to the PN 2.0-LLIN. But, due to the mosquito deterrence as result of excito-reppelency effect, the community protection estimated by the model predicted that the Gen3 T-Net would achieve up to a 13-fold increase in mosquito mortality over the PN2.0. Thus, mass mosquito trapping as a result of a long-term use of T-Nets should lead to a decline in the vector population to a level where transmission is no longer stable. This population decrease is even more likely in the context of insecticide resistance where mosquito mortality is lower than expected by LLINs.

The T-Net exploits the natural behavior of mosquitoes, which principally interact with the roof of the mosquito net[13,14]. Therefore, the positioning of the traps on the top of the net promotes entrapment of mosquitoes. This is especially true for insecticide-free T-Nets which do not cause repellent effects that conventional LLINs create. Untreated T-Nets can therefore be an asset for vector control as they trap and kill mosquitoes irrespective of their insecticide resistance status. Nevertheless, the addition of a trap compartment to existing LLIN can enhance the killing performance of the net (data not shown). Hence, if the idea of using an insecticide-free tool for malaria vector control is far from

being practiced, an interim solution could be to use a hybrid version of T-Net consisting of an insecticide-free trap compartment mounted on the LLINs currently used. In this case, community protection would be provided both by the insecticidal effect and the mass trapping of mosquitoes.

Numerous studies have shown that the escalation in resistance to insecticides closely matches the introduction of insecticide-treated nets. To combat resistance to insecticides, the trend today is to use LLINs combined with the synergist piperonyl-butoxide (PBO)[24]. The PBO inhibits enzymatic activity of mosquito insecticide detoxification genes, and therefore enhances the insecticidal effect of treated bed-net. Unfortunately, decreases in performance of these mosquito nets are starting to be observed in certain regions of Africa where mosquitoes are resistant[25]. Because the T-Net is insecticide-free, it does not exert any insecticide resistance selection pressure on mosquitoes, and because it is not impacted by insecticide resistance, its exclusive and prolonged use in areas that are endemic for resistance should eventually lead to a decrease in the level of resistance and thus facilitate the reintroduction of insecticides.

Though we have not analyzed the trapped mosquitoes escape rate in the field trial, we have found in laboratory conditions that trapping elicits a mosquito response that rapidly leads to exhaustion and death from dehydration and starvation in one to two days. We also found that trapped dead mosquitoes were completely washed out when the T-Nets were hand-washed as per traditional washing method in Africa. This finding suggests that removing mosquitoes from the T-Net trap compartment is not an issue. They could also be removed by net shaking when the funnels are pulled out. However, we believe that the view of mosquitoes in the trap compartment can be an asset to the extent that it could encourage the use of bed-nets.

Sampling of mosquito populations for transmission studies or evaluation of other vector control tools such as IRS could also be done with insecticide-free T-Nets. Various anthropophagous mosquito sampling tools exist but are complex to build up[25-27]. If compared to these methods, the simple construction mode of T-Net, which uses inexpensive materials, makes it an easy-to-assemble and a less expensive sampling tool. It would be interesting to consider a comparative study of its effectiveness as a sampling method vis-à-vis other methods.

We have not conducted in depth manufacturing cost studies, yet improvements for mass production at low cost are underway. However, we anticipate a satisfactory cost-effectiveness ratio and the ability to stay in the range of 2 to 4 dollars[28] per T-Net, which is the current range of LLINs cost on the market. Furthermore, because there is no new chemistry to develop, the route to market should be rapid compared to an insecticide treated bed-net.

Materials and Methods

Tunnel test for assessment of knitted cone efficency

A modified WHO-tunnel[17] test experiment was conducted to assess the rate at which mosquitoes would move through the knitted cones. The tunnel was modified to include three compartments: the top compartment which operated as a mosquito release chamber, a middle compartment which operated as a trapping container, and a lower compartment that contained the arm of a human subject to attract host-seeking mosquitoes. The top and middle compartment were separated by the knitted cone being tested, and the middle and lower compartment were separated by a piece of bed-net fabric to prevent the human subject from receiving mosquito bites.

The tunnel was placed vertically to mimic the position of a cone on a T-Net. A human arm was introduced in the bottom compartment. Two replicates of 50 and 106 unfed five to eight-day old host seeking *An. gambiae* Kisumu female mosquitoes were released into the top compartment. After 1.5 h, the mosquitoes that remained in the release chamber were removed using a mouth aspirator and counted. The human arm was removed from the bottom compartment, and trapped mosquitoes were observed for an additional 2 h. Thereafter, the top compartment was checked for any egression (mosquito passage from the trap compartment to the top compartment). The trapped mosquitoes were then counted.

Gen3 T-Net proof of concept investigation: laboratory walk-in cage trial

Laboratory walk-in cage trials were conducted in the Dearstyne Entomology Building in the Department of Entomology and Plant Pathology at NCSU in a walk-in sized bioassay cage (1.58 x 2.18 x 2.03 m, depth x width x height) at 27$\pm$1ºC; 65$\pm$4% RH using the Gen3 non-insecticide T-Net. Trapping was conducted with a human subject sleeping under the net (NCSU IRB approved protocol 9067). Five to eight-day old unfed *An. gambiae* Kisumu-strain adult mosquitoes reared at Dearstyne Laboratory according to MR4 rearing protoco[29]. The mosquitoes were released in six replicates of 41 to 106 into the walk-in cage after the human subject had entered the sleeping net compartment. Trapping was conducted for 1.5 hours during the photophase, since this mosquito strain for years has been fed with lights-on. At the end of the experiment, mosquitoes were vacuum-collected from outside of the trap compartment in the walk-in cage and counted immediately. To assess eggression, trapped mosquitoes were not removed from the trap compartment immediately. They were counted immediately after the end of experiment, and after 30 minutes, 1 hour, 2 hours, 6 hours and overnight. They were removed from the trap compartment the following day. The study was conducted in six different days between in April 2018. The mosquitoes were obtained

from MR4-BEI Resources, and with current NCSU biosafety approval, a continuous colony was maintained in our laboratory using methods previously described.

Field study

Study site

The study was conducted in experimental huts according to WHO protocol[17] in the municipality of Tiassalé (5°53'54" N et 4°49'42" W) in November 2018. The site is located in the south of Côte d'Ivoire at about 110 km North of the country's major city Abidjan. The climate is tropical and characterized by four seasons. A long rainy season (March-July) during which two thirds of the annual rain falls, a short dry season (July-August), a short rainy season (September-November) and a long dry season (December to March). The average annual rainfall is 1,739 mm with an average annual temperature of 26.6 °C. The annual average relative humidity is around 70%. Rice production in the lowlands of Tiassalé which facilitates the proliferation of mosquitoes throughout the year, and malaria is the leading cause of morbidity in the local population. The transmission of the disease is mainly due to *An. coluzzii* (80%) and *An. gambiae* (20%) which have developed multiple resistance to insecticides[19,20.]

Experimental station

The experimental field station of Tiassalé is made of 18 standardized experimental huts (Fig. 1a) situated close to the rice plantations. Each hut is 2.5 m long, 1.75 m wide and 2 m high. The walls are made of concrete bricks and plastered with cement, while the floor is made of cement and the roof of corrugated iron sheets. A plastic cover is mounted underneath the roof as a ceiling to facilitate manual collection of mosquitoes. Each hut is built on a platform made of concrete surrounded by a water-filled moat that prevents entry of foraging ants. Entry of mosquitoes is facilitated through four 1 cm wide window slits located on three sides of the hut. The slits are designed in such a way as to prevent mosquitoes from escaping once they are inside the hut. Each hut is equipped with a veranda trap located on the fourth side, made of sheeting and screening mesh to capture mosquitoes that would otherwise escape.

Mosquito collection and processing

Four sleepers were trained and paid to sleep under the bed-nets randomly allocated to four huts from 9.00 pm to 5.00 am and to collect mosquitoes the subsequent morning. Sleepers and bed-nets rotated on daily basis according to three complete Latin square table (extended data) and two additional nights, with the exception of the weekend. Thus, the trial ran for 14 days. Each day after the completion of collections, the huts were cleaned, and the bed-nets rotated appropriately. Prior to the trial, sleepers were all vaccinated against yellow fever and were taken to the hospital to check for malaria

parasites. All sleepers gave informed consent prior to enrolment in the study (CSRS IRB protocol #02-2011/MSLS/CNER-P). Within the hut, resting and dead non-trapped mosquitoes were collected using 5 ml, 12 mm x 75 mm glass hemolysis tubes from the room and in the veranda trap. Trapped mosquitoes were removed with mouth aspirators from the T-Net trap compartment by field technicians. All mosquitoes were taken to the field laboratory and identified to genus level and scored as trapped-dead or trapped-alive, not trapped-dead and not trapped-alive (extended data). Live mosquitoes were placed in cups and given access to sugar solution for 24 h in the insectary at 25-27°C and 70-80% relative humidity (RH) to assess delayed mortality.

Study arms

Three different insecticide-free T-Nets (Gen1, Gen2 and Gen3, Fig. 6) were evaluated in comparison to the WHO recommended and widely used Permanet 2.0-LLIN (PN2.0) used as positive control. The PN2.0 was brand new and provided by the National Malaria Control program from Cote d'Ivoire.

Data analysis

The Kruskal-Wallis non-parametric test with the Conover-Iman multiple pairwise comparisons and the Bonferroni correction were used to analyze the field data. The precision was fixed at 5%. Analysis was done using the XLSTAT software package version 2019.4.1.[30] The overall blood-feeding rate was less than 3%; thus, this parameter was not evaluated.

List of abbreviation

T-Net: Trapping bed-net; Min: minute; WHO: World Health Organization; LLIN: long lasting insecticidal net; PN2.0: Permanet 2.0; Gen: generation; NCSU: North Carolina State University; Std dev.: standard deviation.

Acknowledgments

This work was supported by Innovation Vector Control Consortium (IVCC).

Authors' contributions

CSM initiate the T-Net conception, contributed to design Gen-1, Gen-2 and Gen-3 T-Nets, carried out laboratory studies, supervised experimental field trials, analysed the data and drafted the manuscript. KL proposed and verified the T-Net model. AW designed and conceived the knitted cones. BKF helped supervising the field study. MMC, MR, CA

worked to design the T-Nets, approved the study design, edited and improved the manuscript. All authors read and approved the final manuscript.

Ethical Approval and Consent to participate

Approval for the research study in experimental huts in Cote d'Ivoire was obtained from the National Ethics Committee (CSRS IRB protocol #02-2011/MSLS/CNER-P). All the sleepers provided written informed consent to participate.

Consent for publication

All the participants consented for publication**.**

Availability of data

All data generated or analysed during this study are included in this published article.

Extended data

Crude field data and other supplementary information are included on extended data file.

Competing interest

No competing interests were disclosed.

1. World malaria report 2016. (2016).
2. Global plan for insecticide resistance management in malaria vectors. (World Health Organization, 2012).
3. Bhatt, S. *et al.* The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. *Nature* **526**, 207–211 (2015).
4. Ranson, H. & Lissenden, N. Insecticide Resistance in African Anopheles Mosquitoes: A Worsening Situation that Needs Urgent Action to Maintain Malaria Control. *Trends in Parasitology* **32**, 187–196 (2016).
5. Müller, P. *et al.* Pyrethroid tolerance is associated with elevated expression of antioxidants and agricultural practice in Anopheles arabiensis sampled from an area of cotton fields in Northern Cameroon: INSECTICIDE TOLERANCE IN ANOPHELES ARABIENSIS. *Molecular Ecology* **17**, 1145–1155 (2007).
6. Edi, C. V. A., Koudou, B. G., Jones, C. M., Weetman, D. & Ranson, H. Multiple-Insecticide Resistance in *Anopheles gambiae* Mosquitoes, Southern Côte d'Ivoire. *Emerg. Infect. Dis.* **18**, 1508–1511 (2012)
7. Strode, C., Donegan, S., Garner, P., Enayati, A. A. & Hemingway, J. The Impact of Pyrethroid Resistance on the Efficacy of Insecticide-Treated Bed Nets against African Anopheline Mosquitoes: Systematic Review and Meta-Analysis. *PLoS Med* **11**, e1001619 (2014).
8. Hemingway, J. The role of vector control in stopping the transmission of malaria: threats and opportunities. *Phil. Trans. R. Soc. B* **369**, 20130431 (2014).
9. Ranson, H. *et al.* Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends in Parasitology* **27**, 91–98 (2011).
10. https://www.ivcc.com/research-development/insecticide-discovery-and-development/
11. Mouhamadou, C. S. *et al.* Evidence of insecticide resistance selection in wild Anopheles coluzzii mosquitoes due to agricultural pesticide use. *Infect Dis Poverty* **8**, 64 (2019).
12. Van Loon, J. J. A. *et al.* Mosquito Attraction: Crucial Role of Carbon Dioxide in Formulation of a Five-Component Blend of Human-Derived Volatiles. *J Chem Ecol* **41**, 567–573 (2015).
13. Parker, J. E. A. *et al.* Infrared video tracking of Anopheles gambiae at insecticide-treated bed nets reveals rapid decisive impact after brief localised net contact. *Sci Rep* **5**, 13392 (2015).
14. Sutcliffe, J., Ji, X. & Yin, S. How many holes is too many? A prototype tool for estimating mosquito entry risk into damaged bed nets. *Malar J* **16**, 304 (2017).
15. Hernandez, H. Standard Maxwell-Boltzmann distribution: Definition and Properties. (2017) doi:10.13140/RG.2.2.29888.74244.
16. Darriet, F. & N'Guessan, R. Un outil expérimental indispensable à l'évaluation des insecticides : les cases-pièges. *Bull Soc Pathol Exot* 6 (2002).
17. WHO_CDS_WHOPES_GCDPP_2005.11.pdf.
18. Chouaïbou, M. S. *et al.* Influence of the agrochemicals used for rice and vegetable cultivation on insecticide resistance in malaria vectors in southern Côte

d'Ivoire. *Malar J* **15**, 426 (2016). Fodjo, B. K. *et al.* Insecticides Resistance Status of *An. gambiae* in Areas of Varying Agrochemical Use in Côte D'Ivoire. *BioMed Research International* **2018**, 1–9 (2018).

19. Fodjo, B. K. *et al.* Insecticides Resistance Status of *An. gambiae* in Areas of Varying Agrochemical Use in Côte D'Ivoire. *BioMed Research International* **2018**, 1–9 (2018).
20. Menze, B. D. et al. An Experimental Hut Evaluation of PBO-Based and Pyrethroid-Only Nets against the Malaria Vector Anopheles funestus Reveals a Loss of Bed Nets Efficacy Associated with GSTe2 Metabolic Resistance. *Genes* **11**, 143 (2020).
21. Ngufor, C. *et al.* Combining Organophosphate Treated Wall Linings and Long-lasting Insecticidal Nets for Improved Control of Pyrethroid Resistant Anopheles gambiae. *PLoS ONE* **9**, e83897 (2014).
22. Russell, T. L., Beebe, N. W., Cooper, R. D., Lobo, N. F. & Burkot, T. R. Successful malaria elimination strategies require interventions that target changing vector behaviours. *Malaria Journal* **12**, 56 (2013).
23. Sougoufara, Seynabou, et al. "Challenges for malaria vector control in sub-Saharan Africa: Resistance and behavioral adaptations in anopheles populations." *Journal of Vector Borne Diseases*, vol. 54, no. 1, 2017, p. 4. *Gale Academic Onefile*, Accessed 30 Jan. 2020.
24. Toé, K. H. *et al.* Increased Pyrethroid Resistance in Malaria Vectors and Decreased Bed Net Effectiveness, Burkina Faso. *Emerg. Infect. Dis.* **20**, (2014).
25. Govella, N. J. *et al.* A new tent trap for sampling exophagic and endophagic members of the Anopheles gambiae complex. *Malar J* **8**, 157 (2009).
26. LaBrecque, B. *et al.* Sampling Host-Seeking Anthropophilic Mosquito Vectors in West Africa: Comparisons of an Active Human-Baited Tent-Trap Against Gold Standard Methods. *The American Journal of Tropical Medicine and Hygiene* **92**, 415–421 (2015).
27. Tangena, J.-A. A., Thammavong, P., Hiscox, A., Lindsay, S. W. & Brey, P. T. The Human-Baited Double Net Trap: An Alternative to Human Landing Catches for Collecting Outdoor Biting Mosquitoes in Lao PDR. *PLoS ONE* **10**, e0138735 (2015).
28. https://www.unicef.org/supply/files/Long-Lasting_Insecticidal_Nets_price_data_January_2018.pdf
29. Benedict, M. Q. Methods in Anopheles research. Malaria Research and Reference Reagent Resource Center (MR4) (2007).
30. Addinsoft (2020). XLSTAT 2019.4.1 statistical and data analysis solution. New York, USA. https://www.xlstat.com