Sub-lethal insecticide exposure affects host biting efficiency of *Kdr*-resistant *Anopheles gambiae*

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Abstract

The massive use of insecticide-treated nets (ITNs) has drastically changed the environment for malaria vector mosquitoes, challenging their host-seeking behaviour and biting success. Here, we investigated the effect of a brief exposure to an ITN on the biting behaviour of *Anopheles gambiae* mosquitoes and the interaction between such behaviour and the *kdr* mutation that confers resistance to pyrethroids. To this aim, we developed a video assay to study the biting behaviour of mosquitoes with similar genetic background, but different *kdr* locus genotypes (SS i.e. homozygous susceptible, RS i.e. heterozygous and RR i.e. homozygous resistant), after a brief exposure to either control untreated nets or one of two types of pyrethroid-treated nets (deltamethrin or permethrin). In presence of untreated nets, the *kdr* mutation did not influence mosquito blood feeding success but caused differences in feeding and prediuresis durations and blood meal size. Exposure to deltamethrin ITN
decreased the blood feeding success rate of RR and RS mosquitoes, whereas permethrin ITN increased the blood-feeding success of RR mosquitoes. Exposure to the two types of pyrethroid-treated nets reduced feeding duration, prediuresis duration and blood meal size of all three genotypes. We discussed the potential consequences of the observed behavioural changes on malaria vector fitness and disease transmission.

**Keywords:** pyrethroid insecticides, long-lasting treated nets, malaria, probing, blood meal, prediuresis;

**Introduction**

Malaria vector mosquitoes become infected by and then can transmit *Plasmodium spp.* parasites during blood meals. *Plasmodium spp.* transmission is strongly dependent on the mosquito biting rate on humans, as formalized by the Ross-MacDonald model of malaria [1]. The behaviour and host preferences of blood-feeding mosquitoes are influenced by several factors, primarily their genetic background [2–5], but also environmental factors, such as host diversity and availability [6,7], and the presence/absence of physical and chemical barriers, such as Insecticide-Treated Nets (ITNs) [8–10].

ITNs should hamper the contacts between humans and nocturnal, anthropophilic and endophagic mosquitoes, such as *An. gambiae* [11]. The widespread use of pyrethroid (PYR) insecticides in public health (i.e. ITNs or indoor residual sprayings), crop protection and other selective pressures (such as xenobiotics) drove adaptations in malaria vectors to reduce the insecticidal effect [12–16].

Physiological PYR resistance involves two main mechanisms: (i) metabolic resistance, due to quantitative or qualitative changes in detoxification enzymes (cytochrome P450 monooxygenases, esterases and glutathione S-transferases), and (ii) target site resistance, due
to non-synonymous mutations in the voltage-gated sodium channels that are called knock-down resistance (kdr) mutations [12,17].

Mosquitoes can reduce vector control tools efficacy also through behavioural adaptations. In areas of sub-Saharan Africa where large-scale vector control programmes have been implemented, several observations suggest that malaria vectors can avoid contacts with ITNs or insecticides on walls by modulating their host-feeding activity. For instance, following the large scale distribution of ITNs in Benin, some An. funestus populations started to feed predominantly at dawn/early in the morning or in broad daylight [18,19], when most people are outside the nets [19]. Behavioural modulation might also be influenced by physiological resistance mechanisms. Indeed, experimental hut trials showed that in An. gambiae, indirect behavioural indicators in response to ITN presence (deterrence and induced exophily) are related to the physiological tolerance to insecticide [20]. Moreover, a study demonstrated that kdr homozygous resistant mosquitoes have longer contacts with ITNs than homozygous susceptible mosquitoes, which are more excited by PYR irritant effect [21]. Altogether, these findings indicate that insecticide treatments could affect the behaviour of malaria vectors. However, the effects of insecticide exposure and kdr mutations on the biting activity of An. gambiae remain poorly investigated.

The last step of the mosquito host-seeking behaviour after reaching a host protected by an ITN is biting for taking a blood meal. During the host-seeking phase and the penetration through a hole in the net, mosquitoes can be exposed to sub-lethal doses of insecticide [22–24]. Such doses do not cause death, but can have several physiological or behavioural effects on host-seeking mosquitoes [25]. For instance, sub-lethal doses of insecticide can affect the feeding and reproductive behaviour of some blood-sucking insects [26]. As the PYR insecticide on ITNs acts on specific sites in the mosquito nervous system, it might alter some
physiological processes involved in the biting behaviour of malaria vectors. There is currently scarce literature on this subject [27].

In the present study, we investigated whether pre-exposure to an ITN modulates the mosquito ability to take a blood meal by using experimental conditions that mirror the exposure to insecticide occurring when a mosquito passes through an ITN after having located a host. We also assessed whether the kdr mutation (L1014F) modifies blood feeding success and biting behaviour of An. gambiae.

**Methodology**

**Ethical Considerations**

This study was carried out in strict accordance with the recommendations of Animal Care and Use Committee named “Comité d’éthique pour l’expérimentation animale; Languedoc Roussillon” and the protocol was approved by the Committee on the Ethics of Animal Experiments (CEEA-LR-13002 for the rabbits).

**Mosquito strains and rearing procedures**

Two mosquito laboratory strains were used for this study. One is the insecticide-susceptible Kisumu strain (KISUMU1, MRA-762, VectorBase stable ID VBS0000026 on vectorbase.org), isolated in Kenya in 1975. This strain is PYR-susceptible and homozygous (SS) for the L1014 codon. The second one is the Kdrkis strain that is PYR-resistant and homozygous (RR) for the L1014F kdr mutation. The Kdrkis strain was obtained by introgression of the kdr-west allele (L1014F) into the Kisumu genome through 19 successive back-crosses between Kisumu and VKPer [28]. VKPer strain used to obtain Kdrkis displayed the same expression level of metabolic resistance enzyme as Kisumu [29].
Polymorphisms between Kisumu and KdrKis strains are expected to be restricted in the flanking region of the \textit{Kdr} allele (15 cM for 19 backcrossing generations [30,31]) and the observed phenotypes are therefore expected to be associated to this genetic area.

Heterozygous individuals (RS) for the L1014F \textit{kdr} mutation were obtained by crossing once Kisumu SS females (F1 progeny) with Kdrkis RR males. Therefore, the three genotypes have a common genetic background for most of their genome.

Mosquitoes were reared at 27 ± 1°C, 70-80\% relative humidity under a 12h:12h (light : dark) photoperiod in the insectary. Gravid females were allowed to lay eggs on wet filter paper inside mesh-covered cages. Eggs were dispensed in plastic trays containing osmotic water. Larvae were kept in trays and fed with TetraMin\textsuperscript{®} fish food. Pupae were removed and allowed to emerge inside 30x30x30cm cages. After emergence, adults could feed \textit{ad libitum} on 10\% sucrose solution.

On each experimental day, a batch of ten adult (7 to 9-day-old) female mosquitoes never fed with blood were randomly collected from the rearing cages and placed in cups covered by gauze. Mosquitoes were starved the day before the experiment because sucrose inhibits blood avidity in mosquitoes [32].

\textbf{Insecticide exposure}

To simulate the contact with an ITN that may occur before the vector finds a way to reach a host, starved females were individually exposed to PYR insecticide-treated netting using an experimental setup in which insects can only walk or stand on the net surface and that was initially designed to measure the median knock down time [33]. We used three net types (i.e. treatments): an untreated net (negative control), an Olyset Net\textsuperscript{®} (impregnated with 1000 mg/m\textsuperscript{2} of permethrin, hereafter Olyset) and a PermaNet\textsuperscript{®} 2.0 (coated with 55 mg/m\textsuperscript{2} of deltamethrin, hereafter PermaNet). Based on previous experiments [24], each mosquito was
exposed for 30 seconds. This is the median time of contact with permethrin-treated netting
before PYR-susceptible anopheles (SS genotype) locate a hole to reach the host [24]. After
exposure, a 1-min latency period was observed before releasing the insect in the behavioural
assay setup.

**Behavioural assay**

Experiments were conducted in a biting behavioural assay setup designed for video recording
the biting behaviour of the tested mosquitoes (Figure 1). The setup is made of a foam board
and composed of an observation tunnel (OT) and an observation zone (OZ) separated by a
transparent plastic (TP). The OZ is a triangular prism, closed on its base by a removable paper
sheet with a 1-cm diameter hole. The hole allows mosquitoes to bite the ear of a rabbit (R)
that is maintained immobile in a restraining cage (RC) to limit ear movement during blood
feeding. The same rabbit was used during all experiments. The experimental room was faintly
illuminated with a compact fluorescent lamp bulb placed at 15 cm from the OZ to allow the
acquisition of the biting behaviour sequence by the digital video system.

For each trial, a mosquito was individually released inside the behavioural assay through a
circular opening (CO) located on its lateral face. Cotton was used to plug the CO after
releasing. The number of released individual anopheles per genotype and treatment ranged
from 43 to 86. Each mosquito was filmed for 10 minutes using a Sony® Digital HD Video
Camera (HDR-XR550) placed on the top of the observational tunnel (Figure 1). The video
camera was connected to a computer screen placed outside the experimental room to allow
real-time monitoring of each mosquito, to avoid any disturbance of the rabbit and interference
of the experimenter on the mosquito behaviour. The MPEG-2 recordings (PAL video:
720x576 pixels at 25 frames/s) were analysed using the behavioural event recording program
The experimenter used latex gloves to avoid any contamination by human skin odours. The OZ was cleaned with ethanol and the removable paper was changed after each mosquito was tested to avoid any contamination by insecticide residues between behavioural assays.

**Feeding success and behavioural parameters**

A mosquito was scored as successful if it was feed at the end of the 10-min trial (whatever the amount of blood it took) and unsuccessful if it did not. After the trial, successfully fed mosquitoes were stored at -35°C for measuring the blood meal size (see below). As pre-exposure to insecticide can induce a knockdown (KD) effect during the trial, mosquitoes were recorded as KD, if they were lying on their side or their back.

Analysis of the acquired images allowed quantifying the following variables in fed mosquitoes: (1) number of probing events, (2) probing duration (the time from the introduction of the stylet fascicule into the rabbit skin to blood appearance in the mosquito abdomen), (3) feeding duration (from the blood appearance in the abdomen to the beginning of the stylet fascicule withdrawal), and (4) prediuresis duration (the time during which excretion of rectal fluid (plasma, water, metabolic wastes) is observed as red bright drops during feeding and after proboscis withdrawal).

**Blood meal volume measurement**

The blood intake was evaluated by quantifying the haemoglobin amount, as described by Briegel [34]. Each engorged mosquito was stored in one 1.5 ml Eppendorf tube at -35°C. Then, the whole abdomen was ground in the presence of 0.5 ml of Drabkin’s reagent until it was completely disintegrated. Haemoglobin then reacted with the Drabkin’s reagent and was converted into haemoglobin cyanide (HiCN). Samples were incubated at room temperature (25°C) for 20 min and then a chloroform solution was added in each tube. Samples were centrifuged at 5600 rpm for 5 min and the aqueous supernatant (containing HiCN) was placed.
in a new 1.5 ml Eppendorf tube. The absorbance was read at a wavelength of 550 nm using a microplate spectrophotometer. Two replicates were done for each mosquito and their absorbance values were averaged. A sample of the rabbit blood was used as control for calibration curves.

As the blood meal volume is correlated with the mosquito size [35], the blood meal volume of all mosquitoes from the same batch was divided by the average weight of five randomly selected mosquitoes from the same rearing cage. The resulting ratio was expressed in µL of blood per µg of weight and called weighted blood meal volume or blood meal size.

Statistical analysis

All statistical analyses were performed using the R software, version 3.5 [36]. We analysed the feeding success (coded as 1 for fed mosquitoes and 0 for unfed ones) with a binomial logistic mixed-effect model using ‘glmer’ function in the ‘lme4’ package [37]. We analysed the number of probing events with a zero-truncated negative binomial mixed-effect model using function ‘glmmTMB’ in the ‘glmmTMB’ package [38]. We analysed durations (probing, feeding and prepuressis) with a mixed effect Cox proportional hazard model using function ‘coxme’ of the ‘coxme’ package [39]. We analysed weighted blood meal size with a linear mixed effect model using function ‘lmer’ in the ‘lme4’ package. All models included the kdr genotypes (SS, RS or RR), type of pre-exposure (untreated, Olyset or PermaNet) and their interactions as fixed terms explanatory variables and the date of the experiment as a random intercept. We used Tukey’s post-hoc test to perform multiple comparisons among genotypes and treatments using ‘emmeans’ function [40]. We computed Odds Ratios (OR) for the binomial model, Hazard Ratios (HR) for Cox models, Rate Ratios (RR) for the negative binomial model, Mean Differences (MD) for the linear model and their 95% confidence intervals.
We calculated the binomial confidence interval of feeding rates and knock-down rates with the Wilson's score method using the ‘binconf’ command from the ‘Hmisc’ package [41].

Effect of feeding duration on blood meal size and prediuresis duration was analysed using a linear mixed effect model and a mixed effect cox proportional hazard model, respectively. Fixed terms included genotypes and treatment and interactions and date was set as a random intercept. We used function ‘emtrends’ of the ‘emmeans’ package [40] to obtain estimates and 95% confidence intervals of the marginal slopes of the trends (between feeding duration and blood meal size or prediuresis duration) for each genotypes and treatments.

Data and codes used for analyses and figures are available [42].

**Results**

In total, 511 *An. gambiae* females (182 SS, 156 RS and 173 RR) were released individually for the study. The number of mosquitoes released, fed, unfed and knock-downed among genotypes and treatments are shown in Table 1.
Table 1. Numbers of mosquito releases per genotype and treatment

| Genotype | Treatment  | N tested | Fed | | | Unfed | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | KD | Non-KD | KD | Non-KD | |
| SS | Untreated | 86 | 0 | 41 | 0 | 45 | |
| SS | Permethrin | 50 | 5 | 5 | 33 | 7 | |
| SS | Deltamethrin | 46 | 1 | 15 | 18 | 12 | |
| RS | Untreated | 70 | 0 | 40 | 0 | 30 | |
| RS | Permethrin | 43 | 3 | 14 | 11 | 15 | |
| RS | Deltamethrin | 43 | 1 | 9 | 15 | 18 | |
| RR | Untreated | 70 | 0 | 40 | 0 | 30 | |
| RR | Permethrin | 53 | 0 | 41 | 0 | 12 | |
| RR | Deltamethrin | 50 | 4 | 12 | 12 | 22 | |


**Impact of the kdr mutation on feeding success and biting behaviour**

When female mosquitoes were pre-exposed to UTN (i.e., untreated netting), no difference in their feeding success was found among the three genotypes (OR_{RR-SS}=1.47 [0.67, 3.22]; OR_{RR-RS}=1.00 [0.44, 2.25]; Figure 2A).

Analysis of the biting behaviour of successful mosquitoes in absence of insecticide pre-exposure showed that feeding and prediuresis durations were shorter in RR than in both SS and RS mosquitoes (feeding duration: HRR_{RR-SS} = 2.15 [1.26, 3.69]; HRR_{RR-RS} = 2.46 [1.43, 4.23]; Figure 2B; prediuresis duration: HRR_{RR-SS} = 1.94 [1.02, 3.71]; HRR_{RR-RS} = 2.11 [1.11, 4.01]; Figure 2C). The weighted blood meal volume of RR mosquitoes was lower than that of RS mosquitoes (MD_{RR-RS} = 0.99 [0.24, 1.74]; Figure 2D). Number of probing events and probing duration were not significantly different among genotypes (SS, RS and RR) (Tukey’s p-value > 0.05).

**Impact of insecticide exposure on knockdown rates**

Pre-exposure to permethrin or deltamethrin induced 76 % [62.6, 85.7] and 41.3% [28.3, 55.7] knock-down rates in SS mosquitoes, respectively. It induced 32.6% [20.5, 47.5] (14/43) and
37.2% [24.4, 52.1] (14/43) KD rates in RS mosquitoes, respectively (Table 1). Among RR mosquitoes, permethrin pre-exposure did not induce any KD effect, whereas deltamethrin pre-exposure led to 30.2% [20.8, 45.8] (16/50) of KD mosquitoes (Table 1).

**Impact of insecticide pre-exposure on feeding success**

When compared to the UTN condition, pre-exposure to permethrin (Olyset) reduced significantly the feeding success of SS mosquitoes (OR$_{perm-UTN}$=0.28 [0.10, 0.73]; Figure 3A), but not that of RS (OR$_{perm-UTN}$=0.49 [0.19, 1.26]; Figure 3B) or RR mosquitoes (OR$_{perm-UTN}$=2.58 [0.97, 6.84]; Figure 3B). Pre-exposure to deltaxmethrin (PermaNet) reduced significantly the feeding success of RS and RR mosquitoes (OR$_{delta-UTN}$=0.23 [0.08, 0.64] and OR$_{delta-UTN}$=0.35 [0.14 – 0.88]; Figure 3B and 3C), but not of SS mosquitoes (OR$_{delta-UTN}$=0.58 [0.24, 1.43]; Figure 3A).

When comparing the feeding success among genotypes after insecticide pre-exposure, the feeding rate of RR mosquitoes was higher than that of SS and RS mosquitoes after permethrin pre-exposure (OR$_{RR-SS}$=13.82 [4.35, 43.92]; OR$_{RR-RS}$=5.29 [1.76, 15.86], Supplementary Figure 1B), whereas the feeding success of RS mosquitoes was not different than that of SS mosquitoes (OR$_{RS-SS}$=2.61 [0.85, 8.02], Supplementary Figure 1B). In contrast, pre-exposure to deltamethrin did not induce any difference in the feeding success of the three genotypes (OR$_{RR-SS}$=0.891 [0.31, 2.53]; OR$_{RR-RS}$=1.55 [0.51, 4.76]; OR$_{RS-SS}$=0.57 [0.18, 1.80], Supplementary Figure 1C).

**Impact of insecticide exposure on biting behaviour**

After exposure to deltamethrin (PermaNet), feeding duration, prediuresis duration and weighted blood meal size were significantly reduced in SS mosquitoes compared to the UTN
condition (feeding duration: \( \text{HR}_{\text{delta-UTN}} = 4.38 \ [2.09, 9.15]; \) prediuresis: \( \text{HR}_{\text{delta-UTN}} = 5.31 \ [2.11, 13.36]; \) blood meal size: \( \text{MD}_{\text{delta-UTN}} = -1.02 \ [-1.82, -0.23]; \) Figure 4A, 4B and 4C, respectively). A similar trend was observed after exposure to permethrin (Olyset) for feeding duration (\( \text{HR}_{\text{perm-UTN}} = 2.87 \ [1.21, 6.81]; \) Figure 4A) and weighted blood meal volume (\( \text{MD}_{\text{perm-UTN}} = -0.97 \ [-1.85, -0.08]; \) Figure 4C) but not for prediuresis duration (\( \text{HR}_{\text{perm-UTN}} = 2.27 \ [0.92, 5.63]; \) Figure 4B).

Compared to UTN, permethrin and deltamethrin reduced the feeding and prediuresis durations as well as blood meal size of both RS (feeding duration: \( \text{HR}_{\text{perm-UTN}} = 7.42 \ [3.55, 15.53]; \)) \( \text{HR}_{\text{delta-UTN}} = 4.57 \ [1.94, 10.76]; \) Figure 4D; prediuresis duration: \( \text{HR}_{\text{perm-UTN}} = 9.44 \ [3.36, 26.46] \) and \( \text{HR}_{\text{delta-UTN}} = 7.22 \ [2.70, 19.29]; \) Figure 4E, blood-meal size: \( \text{MD}_{\text{perm-UTN}} = -1.97 \ [-3.04, -0.90] \) and \( \text{MD}_{\text{delta-UTN}} = -2.01 \ [-2.97, -1.05]; \) Figure 4F) and RR genotypes (feeding duration: \( \text{HR}_{\text{perm-UTN}} = 2.06 \ [1.20, 3.51] \) and \( \text{HR}_{\text{delta-UTN}} = 3.78 \ [1.83, 7.82]; \) Figure 4G; prediuresis duration: \( \text{HR}_{\text{perm-UTN}} = 1.71 \ [0.80, 3.64] \) and \( \text{HR}_{\text{delta-UTN}} = 9.31 \ [3.57, 24.28]; \) Figure 4H; blood-meal size: \( \text{MD}_{\text{perm-UTN}} = -1.52 \ [-2.23, -0.81] \) and \( \text{MD}_{\text{delta-UTN}} = -1.41 \ [-2.28, -0.54]; \) Figure 4I). Number of probing events and probing duration were not significantly different among treatments (UTN and ITN) (Tukey’s p-value > 0.05).

In absence of pre-exposure to insecticide (UTN), the blood meal size and the prediuresis duration were positively correlated with the feeding duration for all genotypes (supplementary table 1 and 2). With the exception of blood meal size of RS pre-exposed to permethrin, these relationships were not observed when mosquitoes were pre-exposed to insecticides (supplementary table 1 and 2), suggesting a perturbation of processes underlying these correlations.
Discussion

To investigate the influence of ITN exposure on the biting behaviour of *An. gambiae* mosquitoes, we used mosquitoes that share the same genetic background, but for the *kdr* allele locus, and then exposed them to ITN prior to blood feeding.

The blood-feeding success did not differ between the three genotypes in the absence of insecticide exposure. Therefore, the *kdr* mutation was not associated with significant change of blood meal success rate. However, feeding duration and blood meal size were different between genotypes. RR mosquitoes spent less time taking their blood meal than RS and SS mosquitoes. This might confer an advantage as fast feeding reduces the risk to be killed because of the host defensive behaviour [43]. On the other hand, RS mosquitoes took higher blood volumes than RR females. This could improve the completion of oogenesis in RS mosquitoes [44] and increase their fecundity compared to RR. However, large blood meals reduce the flying ability, escape speed and agility required to avoid predators [43,45,46].

These different trades-offs between behavioural traits that might enhance fecundity or survival in the three genotypes are of great interest and deserve further investigations in relation with the ecological and vector control environment. Such trades-offs possibly affect mosquito fitness and may therefore drive not only the evolution of insecticide resistance in mosquitoes but also parasite transmission. For example, a decrease in blood meal duration and size might increase the frequency of multiple feedings and consequently the risk of *Plasmodium* transmission [47]. Similarly, a bigger blood meal size might increase the probability of mosquito infection by gametocytes [48].

Exposure to permethrin and deltamethrin induced opposite outcomes in term of blood feeding success (increase and decrease, respectively) in RR mosquitoes. This opposite effect on the feeding success rate of RR females might be linked to the different chemical properties of
permethrin and deltamethrin that induce two types of bursting activity of sodium channels [49,50]. Type II pyrethroids, such as deltamethrin, further delay the inactivation of the voltage-gated sodium channel and in a less reversible way than type I pyrethroids, such as permethrin [51]. The lower PYR susceptibility of homozygous resistant mosquitoes could lead to their over-stimulation compared to susceptible and heterozygous mosquitoes that are more affected by the toxic effect of such insecticides [52].

In contrast, among females that have been successful in taking a blood meal, the behavioural sequence was altered in the same way by both insecticides. They both induced a decrease of feeding duration and prediuresis duration. As discussed above, short feeding durations and small blood-meal lead us to expect that exposure to insecticide may (i) reduce the risk of the vector to be killed due to the defensive behaviour of the host or due to predators, (ii) reduce the possible number of parasites ingested during one feeding attempt that may be compensated by (iii) the increase of multiple feeding (and therefore the higher risk of human-to-mosquito and mosquito-to-human parasite transmission).

Whatever the genotype, blood meal size and prediuresis duration were correlated to feeding duration in absence of pre-exposure to insecticides. This relationship was, in most cases, no longer observed when mosquitoes were pre-exposed to insecticides indicating that sub-lethal contact with insecticides disrupt physiological processes involved in blood meal intake. Such indirect evidence highlight the real need to decipher more deeply with consequences of sub-lethal contact with insecticides.

Prediuresis duration was substantially reduced in RS and RR females after exposure to ITNs. Prediuresis is an intestinal mechanism that plays a crucial role in protein concentration during feeding [53] and contributes to thermoregulation [54,55]. This perturbation of the pre-diuresis-prediuresis phase by sub-lethal doses of PYR insecticides could lead to toxic
accumulation of metabolic wastes and products of oxidative stress in the haemolymph that might affect the lifespan of mosquitoes. This results suggest that mosquitoes with the \textit{kdr} mutation might be more susceptible to new chemicals that target the mosquito renal system and that are currently developed as an alternative to the currently used insecticides [56].

To conclude, our study demonstrates a complex interaction between insecticide exposure and the \textit{kdr} mutation on the biting behaviour of mosquitoes. The behavioural modulation induced by PYR-treated nets also raises concerns about the consequences of the \textit{kdr} resistance-insecticide interaction. In previous studies, we evidenced that RR mosquitoes prefer a host protected by a permethrin-treated net rather than an untreated net [31] and have a remarkable ability to find a hole into a bet net [24]. Herein, we have completed the sequence by showing that permethrin exposure enhances the feeding success of RR mosquitoes. This suggests the whole picture suggests that permethrin ITN may increase vectorial capacity of \textit{An. gambiae} populations in areas where PYR resistance with \textit{kdr} mutation is well established. Insecticide resistance genes in malaria vectors could modify vector competence and the dynamics of infection by \textit{P. falciparum}. For instance, recent studies have shown that parasite infection increases insecticide susceptibility in mosquitoes carrying the \textit{kdr} mutation [57] and that insecticide exposure reduces parasite development in resistant mosquitoes [58]. In addition, malaria parasites have been shown to modify the feeding behaviour of their mosquito vectors in ways that favour their transmission [59–61], but the role of insecticide resistance that could modulate this phenomenon has not been investigated yet [62]. It is therefore urgent to decipher the links between insecticide exposure, resistance mechanisms and infection by \textit{P. falciparum} on the host-seeking and biting behaviour of malaria vectors to better understand malaria transmission in areas where insecticidal tools for malaria prevention are implemented.

All these interactions should then be used as variables to include host-seeking behavioural
modulation by $Kdr$ resistance in models on $P. falciparum$ transmission to better understand and/or predict the efficacy of vector control strategies [63].

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Figure legends

Figure 1 Experimental set-up to monitor the biting behavioural sequence.
OT: Observational tunnel, TP: Transparent plastic, OZ: Observational zone (mosquito biting the rabbit ear), R: Rabbit, RC: Restraining cage

Figure 2 Feeding success and biting behavior in absence of insecticide of Anopheles gambiae females of each kdr genotype.
Feeding success of each genotype and 95% binomial confidence intervals of the proportions (error bars) are shown in panel A. Panels B, C and D show boxes-and-whiskers plots of feeding duration, pre-diuresis duration and blood-meal size, respectively. Boxes indicate 1st-3rd quartile and median values. Whiskers indicate 2.5-97.5 percentiles. Significance according to Tukey’s test after binomial mixed-effect model (panel A), mixed-effect cox proportional hazard model (panel B and C) and after linear mixed effect model (panel D) are indicated (ns p>0.05, * p<0.05, ** p<0.01, *** p<0.001).

Figure 3 Feeding success after exposure to insecticides of Anopheles gambiae females of each kdr genotype.
Feeding success of SS, RS, and RR (panel A, B and C, respectively) genotypes when pre-exposed to untreated, permethrin-treated (Olyset) and deltamethrin-treated (PermaNet) nettings are shown with 95% binomial confidence intervals of the proportions (error bars). Significance according to Tukey’s test after binomial mixed-effect model is indicated (ns p>0.05, * p<0.05, ** p<0.01, *** p<0.001).
Figure 4 Biting behavior after exposure to insecticides of *Anopheles gambiae* females of each *kdr* genotype.

Boxes-and-whiskers plots of feeding duration (panels A, D and G), prediuresis duration (panels B, E and H) and blood-meal size (panels C, F and I) are shown for each genotypes SS (panels A, B and C), RS (panels, D, E and F) and RR (panels G, H and I). Boxes indicate 1st-3rd quartile and median values. Whiskers indicate 2.5-97.5 percentiles. Significance according to Tukey’s test after mixed-effect cox proportional hazard model (panels A, B, D, E, G and H) and after linear mixed effect model (panels D, F and I) are indicated (ns p>0.05, * p<0.05, ** p<0.01, *** p<0.001).

Supplementary Figure 1: Among *kdr* genotypes comparison of feeding success of *Anopheles gambiae* females after exposure to insecticides.

Feeding success of each genotype and 95% binomial confidence intervals of the proportions (error bars) are shown after exposure to untreated net (Panel A, same as Figure 2A), to permethrin treated net (Olyset; panel B) and to deltamethrin treated net (Permanet; panel C). Significance according to Tukey’s test after binomial mixed-effect model is indicated (ns p>0.05, * p<0.05, ** p<0.01, *** p<0.001).
Figure 1
Figure 2
Figure 3

![Graph showing blood-feeding success rate](image)

- **A**: SS
  - **ns**
  - **ns**

- **B**: RS
  - **ns**
  - **ns**

- **C**: RR
  - **ns**
  - ***
  - **ns**
Supp. Figure 1

![Graphs showing blood-feeding success rates with untreated, Permethrin, and Deltamethrin treatments.](image)

- **A** Untreated: ns, ns, ns
- **B** Permethrin: ns, **ns**, ***
- **C** Deltamethrin: ns, ns, ns

Blood-feeding success rate is measured against SS, RS, RR conditions.