

# Non-contact detection of pyrethroids widely used in vector control by Anopheles mosquitoes

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11	
12	Abstract
13	Pyrethroids are the most widely used insecticides to control vector borne diseases including

14 malaria. Physiological resistance mechanisms to these insecticides have been well described, whereas those for behavioral resistance remain overlooked. Field data suggest the presence of 15 spatial sensory detection by Anopheles mosquitoes of the pyrethroid molecules used in 16 insecticide-based control tools, such as long-lasting insecticide nets or insecticide residual 17 spraying, opening the way to the emergence of a wide range of behavioral adaptations among 18 19 malaria vectors. However, the spatial sensory detection of these molecules is controversial and needs to be demonstrated. The goal of this study was to behaviorally characterize the non-20 contact detection of three of the most common pyrethroids used for malaria vector control: 21 22 permethrin, deltamethrin an  $\alpha$ -cypermethrin.

To reach this goal, we recorded the behavior (takeoff response) of *Anopheles gambiae*pyrethroid-sensitive and resistant laboratory strains, as well as field collected mosquitoes from
the Gambiae complex, when exposed to the headspace of bottles containing different doses of

the insecticides at 25 and 35°C, in order to represent a range of laboratory and field
temperatures.

We found the proportion of laboratory susceptible and resistant female mosquitoes that took off was, in all treatments, dose and the temperature dependent. Sensitive mosquitoes were significantly more prone to take off only in the presence of  $\alpha$ -cypermethrin, whereas sensitive and resistant mosquitoes showed similar responses to permethrin and deltamethrin. Fieldcollected mosquitoes of the Gambiae complex were also responsive to permethrin, independently of the species identity (*An. gambiae, An. coluzzi* and *An. arabiensis*) or their genotypes for the *kdr* mutation, known to confer resistance to pyrethroids.

The observed ability of *Anopheles* spp. mosquitoes to detect insecticides without contact could favor the evolution of behavioral modifications that may allow them to avoid or reduce the adverse effect of insecticides and thus, the development of behavioral resistance.

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39 Key words: Spatial detection, pyrethroids, takeoff response, Anopheles mosquitoes

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### 41 Introduction

42 Insecticide-based vector control remains the main component for the prevention of malaria transmission. 43 Since the beginning of the century, large scale implementation of insecticide treated nets (ITNs) and 44 indoor residual spraying (IRS) has resulted in a significant reduction of malaria cases [1]. Pyrethroids 45 are the most frequently and widely used class of insecticides to control malaria vector species 46 (Anopheles spp.), due to their low toxicity shown in mammals and their great efficacy in reducing 47 malaria transmission by decreasing both human-vector contact and mosquito longevity [2]. However, 48 the extensive use of pyrethroids to control pest insects, including in agricultural practices, has favored 49 the selection and spread of insecticide resistance within malaria mosquito populations [3–8]. Thanks to 50 the continuous efforts made in the last decades, researchers have been deciphering the different 51 mechanisms behind physiological insecticide resistance in mosquitoes, such as target site resistance 52 [4,9,10], metabolic resistance [4,11] or cuticular resistance [4,12,13]. Besides physiological resistance 53 mechanisms, the emergence of behavioral resistance –i.e., behavioral adaptations that help mosquitoes 54 to partially or completely overcome the deleterious effects of insecticides- have been recognized and 55 may also play an important role as a mechanism reducing the efficacy of insecticide-based vector control 56 tools [14,15]. While less documented than physiological resistance, several studies evidenced behavioral 57 modifications in Anopheles species subsequent to the implementation of insecticide-based control 58 methods in the field, as for instance: increased mosquito outdoor host-seeking (i.e. spatial avoidance) 59 [16–18], host shifts (i.e. trophic avoidance) [18,19] or biting daily-rhythm shifts (i.e. temporal 60 avoidance) [20–22]. Such behavioral modifications may result from the evolution of either constitutive 61 behavioral resistance traits (i.e., selection of genetic behavioral variants over generations) or inducible 62 behavioral resistance traits (i.e., phenotypic plasticity within a generation) [14]. The latter implies that 63 mosquitoes are able to detect the insecticides through their sensory system and therefore modify their 64 behavior accordingly.

65 Proofs of non-contact pyrethroid sensory detection have been deduced from the avoidance response 66 mosquitoes display against certain pyrethroid molecules, particularly the so called "volatile/volatilized pyrethroids" commonly used as spatial repellents: e.g., metofluthrin, transfluthrin or prallethrin [23– 67 68 26]. However, it is less clear whether other pyrethroids commonly used in IRS and ITNs, such as 69 permethrin, deltamethrin or alpha-cypermethrin, may elicit a behavioral response in mosquitoes before 70 contact (i.e., from a distance). According to their vapor pressure values at  $25^{\circ}$ C: e.g., permethrin = 1.48  $\times$  10<sup>-8</sup> mm Hg, deltamethrin = 9.32  $\times$  10<sup>-11</sup> mm Hg and cypermethrin = 2.5  $\times$  10<sup>-9</sup> mm Hg [27], these 71 72 pyrethroids are considered as semi- or non-volatile molecules, and such low-volatility is not in favor of 73 their remote detection by mosquitoes [28,29]. Nevertheless, insecticides applied for vector control in 74 malaria endemic regions are exposed to higher temperatures, e.g., 35°C-39 °C [30-32]. Such 75 temperatures can increase the vapor pressure values of these compounds and thus their volatility, as 76 predicted by the Clausius-Clapeyron equation. Field studies have provided indirect evidence for the 77 potential spatial detection of these chemical compounds in mosquitoes. For instance, meta-analysis

studies showed that in experimental huts, malaria vector species (Anopheles spp.) movement (i.e., 78 79 entrance and exiting) was affected by the presence of ITNs in the huts [33,34]. Depending on the 80 experimental treatments, the results unveiled both: a deterrent and an attractive effect, related to the 81 presence of ITNs in the huts, suggesting that malaria vectors might be able to detect ITNs from a distance 82 before entering the hut. Another study using a dual-choice olfactometer assay found that knock-down 83 resistant (kdr) homozygous An. gambiae mosquitoes, thus resistant to pyrethroids, were more attracted 84 by a host placed behind an ITN than an untreated net, while the presence of insecticide on the net did 85 not affect the choice of susceptible mosquitoes [35]. In other mosquito genera, a recent study evidenced 86 an avoidance response of *Culex quinquefasciatus* and *Aedes aegypti* to permethrin, deltamethrin and  $\lambda$ -87 cyhalothrin, but only when individuals had been previously exposed (by contact) to the molecules before 88 the test [36]. Overall, these results showing either an attraction or an avoidance of pyrethroid compounds 89 by mosquitoes implies the detection of these molecules from distance. Conversely, other studies 90 investigating Anopheles mosquitoes-insecticide interactions did not find any sign of remote repellency 91 in mosquitoes prior to contact [37-42] and the characterization of the distance for the detection of 92 insecticides used in vector control by the targeted mosquitoes remains to clarified.

93 This study aims to determine the non-contact detection of low volatile compounds (permethrin,
94 deltamethrin and alpha-cypermethrin) in laboratory-reared susceptible and *kdr* resistant *An. gambiae* s.s.
95 at two different temperatures, 25 °C and 35°C, that represent a range of realistic conditions. In addition,
96 in order to generalize the results, we tested non-contact detection in wild caught *An. gambiae*,
97 *An.coluzzii and An.arabiensis* mosquitoes.

- 98 Material and methods
- 99 Mosquito rearing

100 Two reference strains of *An. gambiae sensu stricto* were used in this study. The first is the insecticide-101 susceptible reference strain Kisumu (hereafter called "kis" strain), which originates from a field 102 collection in Kenya in 1953 [43]. The second is the pyrethroid resistant strain "kdrkis", homozygous for 103 the *kdr* mutation. This strain was obtained by introgression of the *kdr*-west allele, harboring the L1014F 104 mutation in the voltage-gated sodium channel gene into the Kisumu genome. The kdr-west allele was 105 obtained from pyrethroid resistant mosquitoes sampled in Valley du Kou, Burkina Faso [44]. Both colonies were reared at the "Vectopôle" insectary at the "Institut de Recherche pour le Développement 106 107 (IRD)" in Montpellier (France) and at the "Institut de Recherche en Sciences de la Santé (IRSS)" in 108 Bobo-Dioulasso (Burkina Faso). Adult mosquitoes were maintained under standard insectary conditions 109 (fed with 10% of honey solution, maintained at 27  $\pm$ 1 °C and 70-80% relative humidity and under a 110 12h:12h light:dark cycle). An. gambiae sensu lato L1 and L2 stage larvae were collected in the field at Soumousso (11° 00' 41" N, 4° 02' 50" W) in Burkina Faso. Larvae were transferred into tubes for 111 112 transportation to the insectary at the IRSS and placed under the same conditions as the laboratory strains. 113 Adult females (fed with sucrose 10%) aged from 4 to 8 days post-emergence were used for the 114 behavioral assays. Species identity was determined for each female by PCR [45] after behavioral tests. 115 Genotyping for kdr mutations known to confer resistance to pyrethroids (kdr West and kdr East) was 116 carried out [44,46] as well as genotyping for Ace-1(R) mutation [47] known to confer resistance to 117 carbamates and organophosphates.

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### 119

### - Chemical solutions used:

Permethrin (Sigma-Aldrich, CAS #: 52645-53-1, product #: 45614, batch #: BCCF2182, purity: 95%), 120 121 deltamethrin (Sigma-Aldrich, product #: 45423, batch #: BCCF1130, CAS #: 52918-63-5, purity: 122 98.6%) and alpha-cypermethrin (Sigma-Aldrich, product #: 45806, batch #: BCCF16154, CAS #: 123 67375-30-8, purity: 98.2%) stock solutions were prepared at 1mg/mL in acetone (Sigma-Aldrich, 124 Product #: 650501, CAS #: 67-64-1, purity: 99.99%). In this study, the dose corresponds to the mass of 125 insecticide in micrograms ( $\mu g$ ) placed into the test bottle during the experiment, as we let the acetone 126 completely evaporate under a chemical hood from 5 min to 30 min depending on the dose. Four or five 127 doses of pyrethroids were tested during the experiments:  $4\mu g$ ,  $40\mu g$ ,  $400\mu g$ ,  $800\mu g$  (absent for permethrin) and 1600µg. Acetone was used as a negative control in the behavioral assays and it was 128 129 treated in a similar manner to the insecticide samples.

131 - Behavioral assay set-up description:

132 In this behavioral assay, we determined whether adult female An. gambiae s.l. detected permethrin, 133 deltamethrin and alpha-cypermethrin without contact based on their behavioral response (i.e., takeoff). 134 Females were exposed to the headspace of varying doses of insecticides disposed at two different temperatures: 25°C and 35 °C. A set-up including a sample bottle containing the dose of pyrethroid to 135 136 be tested was placed into a water bath to adjust the headspace temperature to either 25°C or 35°C (Figure 137 1). Headspace temperature was measured using a digital thermometer (model t110, Testo AG, 138 Germany). A 30s continuous charcoal-purified airflow (0.48L/min) at 0.16 cm<sup>2</sup>/s was circulated by 139 means of a stimulus controller (CS-55, Syntech, Germany), through the sample bottle and directed by 140 polytetrafluoroethylene (PTFE) tubing towards a single female mosquito confined in a cylindrical cage 141  $(10 \times 20 \text{ cm})$  enclosed by mosquito netting. The end of the tube was manually placed (by an operator 142 wearing laboratory coat and nitrile gloves) at approximately 1-2 cm from the female mosquito. 143 Mosquitoes from either a laboratory colony or field collected individuals were tested individually at 144 room temperature ( $25-27\pm1^{\circ}C$ ).

Mosquito behavioral response was recorded as a binary variable (i.e., takeoff: yes/no) during the 30s maximum period of exposure to the airflow. For each replicate, 10 individuals were tested per treatment (insecticide *versus* control at both temperatures). Three to five replicates were carried out depending on the mosquitoes involved (i.e., the two laboratory strains and field mosquitoes). Assays involving permethrin on laboratory strains were carried out in Montpellier, France. Assays involving deltamethrin and alpha-cypermethrin on laboratory and field collected mosquitoes were carried out in Bobo Dioulasso, Burkina Faso.





Figure 1: Schematic set-up of the behavioral assay (a: stimulus controller; b: gas washing bottle
containing activated charcoal; c: water bath; d: sample bottle for insecticide or control solution; e: PTFE
tubing; f: cage containing one mosquito female).

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### 157 - Statistical analysis

All analyses were performed in R v.4.3.1. [48]. Initial analyses found that the proportion of females taking off in the control groups differed according to the insecticide test involved: 12.6% of mosquitoes in the control group took off during the assays testing the effect of permethrin, 29.6% in the control group during the assays for deltamethrin, and 32.5% in the control group during the assays for alphacypermethrin (see Supplementary materials Figure S1 a,b). These differences were temporally and physically confounded with the locations in which tests were made. Consequently, the data associated with each insecticide were analyzed separately.

Within the dataset for each insecticide, analyses found the proportion of females responding in the control treatments varied little among treatments or replicates and did not depend on the strain, temperature or dose treatments involved (see Supplementary materials Figure S2 a-c). Consequently, the control data within each insecticide dataset were pooled together to provide a single estimate for the proportion of mosquitoes responding in the control treatments of each dataset.

170 The proportion of mosquitoes responding to the control treatments in each dataset was used as a baseline171 against which the proportions of mosquitoes responding in the insecticide stimulus treatments were

172 compared (see Supplementary materials Figure S2 d-f). The ratio of the latter to the former estimates 173 the relative risk of whether mosquitoes exposed to the insecticide stimulus were more or less likely to 174 respond than those exposed to the control stimulus. Values of relative risk were calculated for the two 175 temperature treatments exposed to the insecticide stimulus in each replicate. In Figure 2, these data are 176 presented in terms of log(relative risk) where values greater than zero (> 0) indicate mosquitoes were 177 more likely to respond to the insecticide stimulus than to the control stimulus.

178 The log(relative risk) data were analyzed by linear regression models with the log(proportion responding 179 in the insecticide stimulus treatment) as the dependent variable and log(proportion responding in the 180 matching control stimulus treatment) as an offset term. For each dataset, the series of models started 181 with a fully factorial model including all the interactions among the parameters; strain, temperature and 182 dose. Subsequent models sequentially dropped one or more parameters until the null model was reached 183 where only the intercept is estimated. These models were ranked according to Akaike's Information 184 Criterium, AIC [49], so as to identify the 'best' model and 'near' models describing the data nearly as 185 equally well as the 'best' model; that is models with an AIC within a value of two of the 'best' model 186 [51]. As we were interested in the trend of mosquito responses with dose, rather than the response 187 associated with any particular dose, we analyzed dose as a continuous parameter on the log-scale, 188 log(dose). Full details of the models tested can be found in the supplementary materials.

Generalized linear models (GLM) for binomial data from the R package 'lme4' [52] were used to analyze the data from field collected mosquitoes. Two-way models with an interaction term compared the proportions of mosquitoes responding to a stimulus treatment (insecticide vs. control) and according to (i) their species, or (ii) their *kdr*-resistance genotype. Note that mosquitoes were genotyped for species and *kdr*-resistance after being behaviorally tested and it was not always possible to resolve both genotypes for each mosquito, resulting in unequal sample sizes across treatments in (i) and (ii).

195

196

197 Results

198 199

# - An. gambiae laboratory susceptible (kis) and resistant (kdrkis) strains' takeoff response after exposure to permethrin, deltamethrin and alpha-cypermethrin

### 200 headspace

In this behavioral assay, we determined whether *An. gambiae* kis and kdrkis females detected permethrin at distance based on their response (takeoff) when exposed to an airflow downstream to the treatment stimulus (i.e., the headspace of a permethrin solution after evaporation of the solvent or the control solvent after evaporation), with regards to the dose of the insecticide and the temperature of the headspace.

The relative risk ratio comparing the response to permethrin headspace relative to the response to the control headspace is represented in figure 2a. Depending on the insecticide, the temperature and mosquito colony, the response of mosquitoes to the insecticide headspace stimulus was significantly greater than to the control stimulus, from the dose 4 or  $40\mu g$  (95% confidence intervals zone not crossing the 0 axe).

211 The proportion of susceptible and resistant female mosquitoes taking off in response to the permethrin

headspace increased with temperature ( $25^{\circ}$ C vs  $35^{\circ}$ C) (P < 0.001) and dose (4; 40; 400 and 1600 µg) (P

213 < 0.001), whereas the strain of mosquitoes had no effect (P=0.763) (Figure 2a and Table Ia).

The same experiment was performed using deltamethrin as the stimulus treatment and following the same experimental conditions except that a supplementary dose of  $800\mu g$  was tested. The proportion of *An. gambiae* taking off in the presence of the deltamethrin headspace increased with the dose (P<0.001)

and temperature (P < 0.001) but did not depend on the strain (P=0.162) (Figure 2b and Table Ib).

218 The same pattern was observed with alpha-cypermethrin headspace with an increased relative risk of

taking off at the higher temperature (P < 0.001) and as insecticide dose increased (P < 0.001) (Figure 2c,

220 Table Ic). In contrast to permethrin and deltamethrin, alpha-cypermethrin headspace induced different

221 responses according to mosquito strains (P=0.012) with the resistant mosquitoes (kdrkis) being less

responsive than the susceptible mosquitoes (kis) (Table Ic).



226 Figure 2: Relative risk of mosquitoes taking off when exposed to three pyrethroids headspaces vs. their 227 response to the headspace of the control stimulus (acetone). a) The relative risk of An. gambiae kis (50 228 females tested per category, N=800) and kdrkis (40 females tested per category, N=640) taking off 229 depending on dose of permethrin and temperature. b) The relative risk of An. gambiae kis (30 females 230 tested per category, N=600) and kdrkis (30 females tested per category, N=600) taking off depending

231	on dose of deltamethrin and temperature. c) The relative risk of An. gambiae kis (30 females tested per
232	category, N=600) and kdrkis (30 females tested per category, N=600) taking off depending on dose of
233	alpha-cypermethrin and temperature. The Y-axis is on a log-scale. The colored bands are the 95%
234	confidence intervals for the linear regression, when the lower interval does not cross the Y-axis at zero
235	it indicates the response of mosquitoes to the insecticide stimulus was significantly greater than to the
236	control stimulus.
237	
238	Table 1: Analysis of variance (ANOVA) based on log(relative risk) of An. gambiae kis and kdrkis
239	females taking off during exposure to the test bottle headspace at different doses of pyrethroids at
240	25°C or 35°C.
241	P < 0.05 is considered as significant. Df= degrees of freedom; Sum.Sq= sums of squares; Mean.Sq=
242	mean square; F.value= value of F distribution; PrF= probability value of F the statistic.
2/12	

243

### a) Permethrin

	Df	Sum.Sq	Mean.Sq	F.value	PrF
Strain	1	0.009	0.009	0.091	0.763
Temperature	1	0.976	0.976	10.099	0.002
log(dose)	1	7.069	7.069	73.169	< 0.001
Residuals	68	6.570	6.097		

245

b) Deltamethrin

	Df	Sum.Sq	Mean.Sq	F.value	PrF
Strain	1	0.066	0.066	2.011	0.162
Temperature	1	0.500	0.500	15.251	< 0.001
*					
log(dose)	1	2.866	2.866	87.491	< 0.001
Residuals	56	1.835	0.033		

#### 247 c) Alpha-cypermethrin

	Df	Sum.Sq	Mean.Sq	F.value	PrF
Strain	1	0.141	1.141	6.674	0.012
Temperature	1	0.405	0.405	19.141	< 0.001
•					
log(dose)	1	1.598	1.598	75.636	< 0.001
Residuals	56	1.183	0.021		

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249

#### Field An. gambiae sl takeoff response to permethrin exposure

250 251 We tested the takeoff responses of *An. gambiae sl* field mosquitoes exposed to the test bottle headspace 252 containing 1600µg of permethrin at 35°C. A total of 192 individuals were exposed to either a control 253 stimulus or an insecticide stimulus of permethrin.

254 Subsequent identification of the species tested found 48 An. arabiensis, 93 An. coluzzii, 35 An. gambiae 255 ss (Figure 3) and 16 individuals were excluded from the analysis as their species could not be identified. 256 Field female mosquitoes were highly responsive to the presence of insecticide, with a significant 257 increase of females taking off in the presence of permethrin headspace for the three species (Table 2). Genotyping of Ace1 and kdr alleles evidenced the presence of Ace1(R) as well as East and West kdr 258 259 resistance alleles (Supp tables 1-3). Behavioral responses were analyzed only in regards to the kdr260 genotypes as they are related to the tested insecticides. For kdr resistant phenotypes, individuals were sequentially categorized as 'RR' if they were homozygous 'rr' for either kdr-east or kdr-west, and then 261 262 as 'RS' if one of the two loci were heterozygous 'rs' for resistance. Individuals homozygous 'ss' at both 263 loci were categorized as 'SS'. Four individuals could not be categorized due to incomplete genotype data 264 (details in supplementary material table 4). Yet, the behavioral response (Figure 4) was tested with 265 regard to the three categorized kdr phenotypes RR (63 individuals), conferring resistance to pyrethroids, 266 RS (38 individuals), conferring partial resistance [53,54] and SS (87 individuals) kdr phenotype which 267 is not conferring resistance to pyrethroids. The three categories showed a significant response to 268 exposure to insecticide (Table 3).





Figure 3: Proportion of field collected mosquitoes of the Gambiae complex taking-off in response to the test bottle headspace containing the compounds. The numbers written in the bar plot correspond to the numbers of tested individuals. Statistically significant differences in the proportion of mosquitoes taking off are noted by asterisks ('\*\*\*' = p<0.001).

276

Table 2: Analysis of variance based on proportion of *Anopheles gambiae sl* mosquitoes responding to
permethrin/control headspace. Sum.Sq= sums of squares; Df= degrees of freedom; F.value= value of F
distribution.

	Sum.Sq	Df	F.values	Pr(>F)
Spacios	0.560	2	0.322	0.720
Species	0.500	2	0.322	0.729
Stimulus	80.149	1	92.121	0.000
Species:Stimulus	1.107	2	0.636	0.541
Residuals	15.661	18		

**280** P < 0.05 is considered as significant.





283

Figure 4: Proportion of field *An. gambiae sl* flying away in response to permethrin headspace in regards to *kdr* genotypes. The numbers written in the bar plot correspond to the numbers of tested individuals. Statistically significant differences of the proportion of mosquitoes taking off are noted by asterisks ('\*\*\*' = p < 0.001).

288

**Table 3:** Analysis of variance based on proportion of *Anopheles gambiae sl* mosquitoes *kdr* phenotypes
responding to permethrin/control headspaces. Sum.Sq= sums of squares; Df= degrees of freedom;
F.value= value of F distribution.

	Sum.Sq	Df	F.values	Pr(>F)
Kdr resistance	2.455	2	1.446	0.262
Stimulus	91.182	1	107.397	< 0.001
Interaction	0.242	2	0.142	0.868
Residuals	15.282	18		

**292** P < 0.05 is considered as significant.

293

294 Discussion

This study provides evidence that female malaria vector mosquitoes are able to detect the most common pyrethroid molecules currently used in vector control (i.e., permethrin, deltamethrin and alphacypermethrin) without direct contact, regardless of the species or their *kdr* genotype. The response to exposure from a distance to the different insecticide headspaces was significantly driven by two parameters: compound dose and temperature.

300 We found a positive relationship between the dose of insecticide placed in a test bottle and the risk that 301 a mosquito would take off relative to those in a matching control treatment. Depending on the nature of 302 the insecticide and the temperature, mosquitoes displayed a sudden locomotor response (takeoff) even 303 when exposed at the lowest doses tested. In this experiment, the amount of insecticide carried by the 304 airflow reaching the mosquito was not quantified, thus it is difficult to extrapolate to the real conditions 305 found during vector control implementation. Commercially available ITNs utilize greater amounts of 306 insecticide compared to our study. The average surface of mosquito nets available on the market is 307 approximatively 17 m<sup>2</sup>, and for instance, Olyset<sup>®</sup> contains 1000mg/m<sup>2</sup> of permethrin, Permanet<sup>®</sup> 2.0 308 contains 56mg/m<sup>2</sup> of deltamethrin, and 200 mg/m<sup>2</sup> of alpha-cypermethrin are used for the Interceptor<sup>®</sup>. 309 Yet, ITN's are designed for a slow release of the compounds by incorporating them within the fibers, 310 and the actual quantity volatilized at a given time is expected to be much lower than the total content 311 values. Very few studies have measured the quantity of pyrethroid released from ITNs. One of the few 312 studies available detected  $0.021 \mu g/m^3$  of cyfluthrin in the close environment of a treated net [55]. 313 Another study, based on volatile collections inside deltamethrin impregnated nets reported that 314 deltamethrin concentration vary between 0.041  $\mu$ g/m<sup>3</sup> and 0.261  $\mu$ g/m<sup>3</sup> in breathing zone and depending 315 on whether the room air conditions were static or dynamic [56]. Regarding IRS, the recommended doses of insecticides spread on indoor walls are in between 20 and 30 mg/m<sup>2</sup> [57]. In this case, insecticides 316 317 are not captured within the fibers, which suggests that a greater amount of insecticide will be 318 bioavailable and potentially able to volatilize compared to those found on nets. Unfortunately, there are 319 no studies, to our knowledge, that have quantified the insecticides present in the air after IRS 320 implementation. The comparison between the quantity of volatilized insecticide in our experiments and 321 the quantity present in natural conditions issued from common insecticide methods (ITN's and IRS)

remains to be investigated. In any case, the goal of this study was to find evidence for the possible
detection of these insecticides by malaria mosquitoes without contact, and the dose-dependent
behavioral response provides it.

325 The observed behavioral response to insecticide headspace exposure was also temperature-dependent. 326 Higher response rates were observed when the insecticides were exposed at a temperature of 35°C, 327 compared to 25°C. Despite the expected low volatility of the tested insecticides, increasing 328 environmental temperature may facilitate the release of a higher number of molecules, as vapor pressure 329 increases with temperature as estimated by the Clausius-Clapeyron equation. It is worth noting that the 330 mosquitoes in the study were placed at laboratory temperature ( $25\pm1$  °C) but exposed to an airflow 331 passing through a water bath at either 25°C or 35°C. This higher temperature condition could potentially 332 influence not only the volatility of the insecticide but also the mosquito's behavior itself. Interestingly, 333 the probability of a behavioral response to the control airflow remained consistent across different 334 temperatures (Figure S2). This means that temperature of the airflow alone did not significantly impact 335 mosquito behavior. Instead, the insecticide-dependent behavioral responses may relate to the 336 temperature of the mosquito itself after experiencing for some time warm air coming from the airflow. 337 This aligns with an emerging body of research highlighting the effect of temperature on mosquito 338 olfaction [58]. The two hypothesis of an increased volatility and a higher olfactory sensitivity of the 339 mosquito at 35°C compared to 25°C are not mutually exclusive and remain to be investigated.

340 The irritancy effect of pyrethroids after contact has been previously shown to be significantly diminished 341 in kdr-resistant mosquitoes [40,59]. In this experiment, the knock-down mutation did not modify the 342 response rates of mosquitoes exposed to permethrin. However, insecticide-susceptible mosquitoes were significantly more prone to take flight when exposed to alpha-cypermethrin than their kdr resistant 343 344 counterparts. Similar trends were observed when exposed to deltamethrin, though the results did not 345 reach statistical significance. These findings suggest two phenomena: i) a differential behavioral effect depending on the insecticide used, which could be the result of insecticide-specific neurotoxic effects 346 347 (i.e., sodium-channel hyper activation) on mosquitoes carrying a specific kdr mutation (L1014F, in this 348 study); and ii) a sensory receptor-dependent detection mechanism which is not related to the insecticide neurotoxic effect. Interestingly, recent studies in a phylogenetically distant mosquito species, *Aedes aegytpi*, have shown that repellency to pyrethrum (i.e., a natural extract containing different pyrethrins,
from which pyrethroids are inspired) and to some pyrethroids is given by a dual mechanism: through a
specific olfactory receptor present in the antennae of mosquitoes and also by the activation of sodium
channels [60–62]. These potential mechanisms, and their relative contributions, should also be
investigated in *Anopheles* mosquitoes.

355 Expanding our investigation, we examined three major field-collected vector species of the Gambiae 356 complex in Burkina Faso: An. gambiae, An. coluzzii, and An. arabiensis. Remarkably, all three species 357 exhibited takeoff responses to permethrin, irrespective of their kdr resistance status. This suggests that 358 the ability to detect this particular insecticide, and potentially other pyrethroids, may be a common 359 characteristic within this complex of species and could be prevalent among most malaria vector 360 populations in sub-Saharan Africa. However, to confirm the occurrence of this phenomenon, further 361 research is required in distant Gambiae populations and in other major malaria vectors, such as An. 362 funestus or An. stephensi.

363

### 364 Conclusion

365 This study not only provides evidence that malaria mosquitoes are able to detect insecticides without 366 the need to get into direct contact, but unravels some of the variables that may influence such detection. 367 The results contrast with previous experimental studies concluding the absence of distant detection by mosquitoes. The discrepancies between these results and our observations may be due to different 368 369 experimental set up and/or how insecticide was presented to the mosquitoes. Nonetheless, the results of 370 this study highlight that dose and temperature may be critical parameters for the spatial detection of 371 insecticides. Such experimental evidence supporting the non-contact detection of permethrin, 372 deltamethrin, and alpha-cypermethrin by malaria vectors deserves further exploration in natural 373 conditions. The presence of non-contact detection in several species of the Gambiae complex opens the 374 possibility to the potential evolution of a large range of behavioral adaptations against insecticides,

which may impede or reduce the efficacy of vector control interventions and lead to the evolution ofbehavioral resistance.

- 377
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- 381
- **382** Author contributions
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- 384 Funding acquisition: CP, AC, DC; Investigation: SSK, AV; Methodology: SSK, AV, PA, AC, DC;
- 385 Project administration: DFSH, RSY, AC, DC; Visualization: SSK, DC; Validation: NM; Supervision:
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- 393
- 394 References

Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria
 control on *Plasmodium falciparum* in Africa between 2000 and 2015. Nature. 2015 Oct;526(7572):207–
 11.

398 2. WHO. Recommended long-lasting insecticidal nets. World Health Organization- Geneva; 2017.

399 3. Diabate A, Baldet T, Chandre F, Akoobeto M, Guiguemde TR, Darriet F, et al. The role of
400 agricultural use of insecticides in resistance to pyrethroids in *Anopheles gambiae s.l* in Burkina Faso.

- 401 Am J Trop Med Hyg. 2002 Dec;67(6):617–22.
- 402 4. Labbé P, David JP, Alout H, Milesi P, Djogbénou L, Pasteur N, et al. 14 Evolution of
  403 Resistance to Insecticide in Disease Vectors. In: Tibayrenc M, editor. Genetics and Evolution of

404 Infectious Diseases (Second Edition). London: Elsevier; 2017. p. 313–39.

405 5. Nkya TE, Poupardin R, Laporte F, Akhouayri I, Mosha F, Magesa S, et al. Impact of agriculture

406 on the selection of insecticide resistance in the malaria vector *Anopheles gambiae*: a multigenerational
407 study in controlled conditions. Parasit Vectors. 2014 Oct 16;7(1):480.

408 6. Nkya TE, Akhouayri I, Kisinza W, David JP. Impact of environment on mosquito response to
409 pyrethroid insecticides: Facts, evidences and prospects. Insect Biochem Mol Biol. 2013 Apr
410 1;43(4):407–16.

411 7. Ranson H, N'guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in
412 African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol. 2011
413 Feb;27(2):91–8.

8. Ranson H, Lissenden N. Insecticide resistance in african *Anopheles* mosquitoes: A worsening
situation that needs urgent action to maintain malaria control. Trends Parasitol. 2016 Mar 1;32(3):187–
96.

417 9. Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Bergé JB, Devonshire AL, et al.
418 Molecular characterization of pyrethroid knockdown resistance (*kdr*) in the major malaria vector
419 *Anopheles gambiae s.s.* Insect Mol Biol. 1998;7(2):179–84.

420 10. Chandre F, Darriet F, Duchon S, Finot L, Manguin S, Carnevale P, et al. Modifications of
421 pyrethroid effects associated with kdr mutation in *Anopheles gambiae*. Med Vet Entomol. 2000
422 Mar;14(1):81–8.

423 11. Vontas J, Katsavou E, Mavridis K. Cytochrome P450-based metabolic insecticide resistance in
424 Anopheles and Aedes mosquito vectors: Muddying the waters. Pestic Biochem Physiol. 2020 Nov
425 1;170:104666.

426 12. Yahouédo GA, Chandre F, Rossignol M, Ginibre C, Balabanidou V, Mendez NGA, et al.
427 Contributions of cuticle permeability and enzyme detoxification to pyrethroid resistance in the major
428 malaria vector *Anopheles gambiae*. Sci Rep. 2017 Sep 11;7:11091.

429 13. Awolola TS, Oduola OA, Strode C, Koekemoer LL, Brooke B, Ranson H. Evidence of multiple
430 pyrethroid resistance mechanisms in the malaria vector *Anopheles gambiae sensu stricto* from Nigeria.

431 Trans R Soc Trop Med Hyg. 2009 Nov 1;103(11):1139–45.

432 14. Carrasco D, Lefèvre T, Moiroux N, Pennetier C, Chandre F, Cohuet A. Behavioural adaptations
433 of mosquito vectors to insecticide control. Curr Opin Insect Sci. 2019 Aug;34:48–54.

434 15. Gatton ML, Chitnis N, Churcher T, Donnelly MJ, Ghani AC, Godfray HCJ, et al. The
435 importance of mosquito behavioural adaptations to malaria control in Africa. Evol Int J Org Evol. 2013
436 Apr;67(4):1218–30.

437 16. Russell TL, Govella NJ, Azizi S, Drakeley CJ, Kachur SP, Killeen GF. Increased proportions
438 of outdoor feeding among residual malaria vector populations following increased use of insecticide-

- 439 treated nets in rural Tanzania. Malar J. 2011 Apr 9;10:80.
- 440 17. Meyers JI, Pathikonda S, Popkin-Hall ZR, Medeiros MC, Fuseini G, Matias A, et al. Increasing
- 441 outdoor host-seeking in *Anopheles gambiae* over 6 years of vector control on Bioko Island. Malar J.
- 442 2016 Apr 26;15:239.
- 443 18. Bøgh C, Pedersen EM, Mukoko DA, Ouma JH. Permethrin-impregnated bednet effects on
  444 resting and feeding behaviour of lymphatic filariasis vector mosquitoes in Kenya. Med Vet Entomol.
  445 1998;12(1):52–9.
- 446 19. Ndenga BA, Mulaya NL, Musaki SK, Shiroko JN, Dongus S, Fillinger U. Malaria vectors and
  447 their blood-meal sources in an area of high bed net ownership in the western Kenya highlands. Malar J.

**448** 2016 Feb 9;15:76.

- 449 20. Moiroux N, Gomez MB, Pennetier C, Elanga E, Djènontin A, Chandre F, et al. Changes in
- 450 Anopheles funestus biting behavior following universal coverage of long-lasting insecticidal nets in
- 451 Benin. J Infect Dis. 2012 Nov 15;206(10):1622–9.
- 452 21. Taylor B. Changes in the feeding behaviour of a malaria vector, *Anopheles farauti Lav.*,
  453 following use of DDT as a residual spray in houses in the British Solomon Islands Protectorate. Trans
  454 R Entomol Soc Lond. 1975;127(3):277–92.
- 455 22. Thomsen EK, Koimbu G, Pulford J, Jamea-Maiasa S, Ura Y, Keven JB, et al. Mosquito
  456 Behavior Change After Distribution of Bednets Results in Decreased Protection Against Malaria
  457 Exposure. J Infect Dis. 2017 Mar 1;215(5):790–7.
- 458 23. Abdel-Mohdy FA, Fouda MMG, Rehan MF, Aly AS. Repellency of controlled-release treated
  459 cotton fabrics based on cypermethrin and prallethrin. Carbohydr Polym. 2008 Jul 4;73(1):92–7.
- 460 24. Achee NL, Bangs MJ, Farlow R, Killeen GF, Lindsay S, Logan JG, et al. Spatial repellents:
  461 from discovery and development to evidence-based validation. Malar J. 2012 Dec;11(1):164.
- 462 25. Argueta T, Kawada H, Takagi M. Spatial repellency of metofluthrin-impregnated multilayer
  463 paper strip against *Aedes albopictus* under outdoor conditions, Nagasaki, Japan. Med Entomol Zool.
  464 2004 Sep 15;55.
- 465 26. Lee DK. Lethal and repellent effects of transfluthrin and metofluthrin used in portable blowers
  466 for personal protection against *Ochlerotatus togoi* and *Aedes albopictus* (Diptera: *Culicidae*). Entomol
  467 Res. 2007;37(3):173–9.
- 468 27. Laskowski DA. Physical and Chemical Properties of Pyrethroids. In: Ware GW, editor. Reviews
  469 of Environmental Contamination and Toxicology [Internet]. New York, NY: Springer New York; 2002
- 470 [cited 2022 Mar 31]. p. 49–170. (Reviews of Environmental Contamination and Toxicology; vol. 174).
- 471 Available from: http://link.springer.com/10.1007/978-1-4757-4260-2\_3
- 472 28. EU REACH Regulation Services. Exploring your options and meeting obligations under the473 European Union's REACH Regulation (Registration, Evaluation, and Authorisation of Chemical

- 474 Substances EC No. 1907/2006) [Internet]. Yordas Group. Available from:
  475 https://www.yordasgroup.com/global-notifications/eu-reach
- 476 29. US EPA. Second peer review of glyphosate [Internet]. 1991. Available from:
  477 https://www3.epa.gov/pesticides/chem search/cleared reviews/csr PC-103601 30-Oct-91 265.pdf
- 478 30. Blanford JI, Blanford S, Crane RG, Mann ME, Paaijmans KP, Schreiber KV, et al. Implications
- 479 of temperature variation for malaria parasite development across Africa. Sci Rep. 2013 Feb 18;3:1300.
- 480 31. Craig MH, Snow RW, le Sueur D. A climate-based distribution model of malaria transmission
- 481 in sub-Saharan Africa. Parasitol Today Pers Ed. 1999 Mar;15(3):105–11.
- 482 32. Paaijmans KP, Imbahale SS, Thomas MB, Takken W. Relevant microclimate for determining
  483 the development rate of malaria mosquitoes and possible implications of climate change. Malar J. 2010
  484 Jul 9;9(1):196.
- 485 33. Moiroux N, Chandre F, Hougard JM, Corbel V, Pennetier C. Remote effect of insecticide486 treated nets and the personal protection against malaria mosquito bites. PloS One. 2017;12(1):0170–
  487 732.
- 488 34. Sherrard-Smith E, Griffin JT, Winskill P, Corbel V, Pennetier C, Djénontin A, et al. Systematic
  489 review of indoor residual spray efficacy and effectiveness against Plasmodium falciparum in Africa. Nat
  490 Commun. 2018 Nov 26;9(1):4982.
- 491 35. Porciani A, Diop M, Moiroux N, Kadoke-Lambi T, Cohuet A, Chandre F, et al. Influence of
  492 pyrethroïd-treated bed net on host seeking behavior of *Anopheles gambiae s.s* carrying the *kdr* allele.
- +52 pyreamore reaced bed net on nost seeking behavior of *micpheles gamblae* s.s earlying the *nar* ancie.
- 493 PloS One [Internet]. 2017 Jul 31;12(7). Available from: https://pubmed.ncbi.nlm.nih.gov/28759566/
- Sougoufara S, Yorkston-Dives H, Aklee NM, Rus AC, Zairi J, Tripet F. Standardised bioassays
  reveal that mosquitoes learn to avoid compounds used in chemical vector control after a single sublethal exposure. Sci Rep. 2022 Feb 17;12(1):2206.
- 497 37. Parker JEA, Angarita Jaimes NC, Gleave K, Mashauri F, Abe M, Martine J, et al. Host-seeking
  498 activity of a Tanzanian population of *Anopheles arabiensis* at an insecticide treated bed net. Malar J.
  499 2017 Jul 4;16(1):270.
- 500 38. Parker JEA, Angarita-Jaimes N, Abe M, Towers CE, Towers D, McCall PJ. Infrared video
- tracking of *Anopheles gambiae* at insecticide-treated bed nets reveals rapid decisive impact after brief
  localised net contact. Sci Rep. 2015 Sep 1;5(1):13392.
- 503 39. Achee NL, Sardelis MR, Dusfour I, Chauhan KR, Grieco JP. Characterization of spatial
- repellent, contact Irritant, and toxicant chemical actions of standard vector control compounds. J Am
  Mosq Control Assoc. 2009 Jun;25(2):156–67.
- 506 40. Deletre E, Martin T, Duménil C, Chandre F. Insecticide resistance modifies mosquito response
  507 to DEET and natural repellents. Parasit Vectors. 2019 Mar 12;12(1):89.
- 508 41. Deletre E, Chandre F, Barkman B, Menut C, Martin T. Naturally occurring bioactive compounds

- from four repellent essential oils against *Bemisia tabaci* white flies. Pest Manag Sci. 2015;72(1):179–
  89.
- 511 42. Deletre E, Martin T, Campagne P, Bourguet D, Cadin A, Menut C, et al. Repellent, irritant and
- 512 toxic effects of 20 plant extracts on adults of the malaria vector *Anopheles gambiae* mosquito. PLOS

513 ONE. 2013 Dec 23;8(12):e82103.

- 514 43. Shute GT. A method of maintaining colonies of East African strains of *Anopheles gambiae*. Ann
- 515 Trop Med Parasitol. 1956 Mar;50(1):92–4.
- 516 44. Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Bergé JB, Devonshire AL, et al.
- 517 Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector
- 518 Anopheles gambiae s.s. Insect Mol Biol [Internet]. 1998 [cited 2022 Nov 26];7(2). Available from:
- 519 https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-2583.1998.72062.x
- 520 45. Santolamazza F, Mancini E, Simard F, Qi Y, Tu Z, della Torre A. Insertion polymorphisms of
- 521 SINE200 retrotransposons within speciation islands of *Anopheles gambiae* molecular forms. Malar J.
  522 2008 Aug 25;7:163.
- 46. Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH. Identification of a point
  mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with
  resistance to DDT and pyrethroids. Insect Mol Biol. 2000 Oct;9(5):491–7.
- 526 47. Bass C, Nikou D, Vontas J, Williamson MS, Field LM. Development of high-throughput real527 time PCR assays for the identification of insensitive acetylcholinesterase (ace-1R) in *Anopheles*528 *gambiae*. Pestic Biochem Physiol. 2010;96:80–5.
- 48. R Core Team. A Language and Environment for Statistical Computing\_. R Foundation for
  Statistical Computing, Vienna, Austria [Internet]. 2023. Available from: https://www.R-project.org/
- 49. Akaike H. Information Theory and an Extension of the Maximum Likelihood Principle. In:
- 532 Parzen E, Tanabe K, Kitagawa G, editors. Selected Papers of Hirotugu Akaike [Internet]. New York,
- 533 NY: Springer; 1998 [cited 2023 Dec 20]. p. 199–213. (Springer Series in Statistics). Available from:
- 534 https://doi.org/10.1007/978-1-4612-1694-0 15
- 535 50. Hurvich CM, Tsai CL. Regression and time series model selection in small samples. Biometrika.
  536 1989 Jun 1;76(2):297–307.
- 537 51. Burnham KP, Anderson DR, editors. Model Selection and Multimodel Inference [Internet]. New Available 538 York, NY: Springer New York; 2004 [cited] 2023 Dec 18]. from: 539 http://link.springer.com/10.1007/b97636
- 540 52. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using Ime4. J
- 541 Stat Softw [Internet]. 2015 [cited 2023 Feb 1];67(1). Available from: http://www.jstatsoft.org/v67/i01/
- 542 53. Zoh DD, Ahoua Alou LP, Toure M, Pennetier C, Camara S, Traore DF, et al. The current
- 543 insecticide resistance status of Anopheles gambiae (s.l.) (Culicidae) in rural and urban areas of Bouaké,

- 544 Côte d'Ivoire. Parasit Vectors. 2018 Mar 2;11:118.
- 545 54. Nguiffo-Nguete D, Mugenzi LMJ, Manzambi EZ, Tchouakui M, Wondji M, Tekoh T, et al.
- 546 Evidence of intensification of pyrethroid resistance in the major malaria vectors in Kinshasa, Democratic

547 Republic of Congo. Sci Rep. 2023 Sep 7;13(1):14711.

548 55. Bomann W. How safe are pyrethroid-treated mosquito nets? An evaluation based on the 549 example of Solfac EW 050. Public Health. 1995;12:30–5.

- 550 56. Vijayakumar A, Ramesh A. Determination of insecticide residues in the air at the breathing zone
- 551 while exposure the long lasting insecticidal net using Gas chromatography with mass spectrometry -
- 552 (GC-MS/MS). Appl Ecol Environ Sci. 2022 Jun 2;10(6):322–7.

553 57. WHO. Indoor residual sprayng: an operational manual for indoor residual spraying (IRS) for
554 malaria transmission control and elimination. World health organization; 2015. Report No.: Second
555 edition.

- 556 58. Lahondère C, Vinauger C, Liaw JE, Tobin KKS, Joiner JM, Riffell JA. Effect of temperature
  557 on mosquito olfaction. Integr Comp Biol. 2023 Aug 1;63(2):356–67.
- 558 59. Chandre F, Darriet F, Duchon S, Finot L, Manguin S, Carnevale P, et al. Modifications of
  pyrethroid effects associated with kdr mutation in *Anopheles gambiae*. Med Vet Entomol.
  560 2000;14(1):81-8.
- 561 60. Liu F, Wang Q, Xu P, Andreazza F, Valbon WR, Bandason E, et al. A dual-target molecular
  562 mechanism of pyrethrum repellency against mosquitoes. Nat Commun. 2021 May 5;12:2553.
- Andreazza F, Valbon WR, Wang Q, Liu F, Xu P, Bandason E, et al. Sodium channel activation
  underlies transfluthrin repellency in *Aedes aegypti*. PLoS Negl Trop Dis. 2021 Jul;15(7):e0009546.
- 565 62. Valbon W, Andreazza F, Oliveira EE, Liu F, Feng B, Hall M, et al. Bioallethrin activates specific
  566 olfactory sensory neurons and elicits spatial repellency in *Aedes aegypti*. Pest Manag Sci.
  567 2022;78(2):438–45.







