

RESEARCH ARTICLE

Spatial distribution and insecticide resistance profile of *Aedes aegypti* and *Aedes albopictus* in Douala, the most important city of Cameroon

Aurelie P. Yougang^{1,2}*, Christophe R. Keumeni^{1,2}, Theodel A. Wilson-Bahun^{1,3}, Armel N. Tedjou^{1,2}, Flobert Njiokou^{1,2}, Charles Wondji^{1,4}, Basile Kamgang^{1,4}

1 Centre for Research in Infectious Diseases, Yaoundé, Cameroon, **2** Faculty of Science, Parasitology and Ecology Laboratory, Department of Animal Biology and Physiology, University of Yaoundé 1, Yaoundé, Cameroon, **3** Faculty of Science and Technology, Laboratory of Vertebrate and Invertebrate Bioecology, Marien-Ngouabi University, Brazzaville, Congo, **4** Vector Biology Department, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

* These authors contributed equally to this work.

* aurelie.yougang@crd-cam.net (APY); basile.kamgang@crd-cam.net (BK)



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Abstract

Prevention and control of *Aedes*-borne viral diseases such as dengue rely on vector control, including the use of insecticides and reduction of larval sources. However, this is threatened by the emergence of insecticide resistance. This study aimed to update the spatial distribution, the insecticide resistance profile of *A. aegypti* and *A. albopictus* and the potential resistant mechanisms implicated in the city of Douala. Immature stages of *Aedes* were collected in August 2020 in eight neighbourhoods in Douala and reared to adult stages. Adult bioassays, and piperonyl butoxide (PBO) synergist assays were carried out according to World Health Organization recommendations. Expression of some candidate metabolic genes including *Cyp9M6F88/87*, *Cyp9J28a*, *Cyp9J10* and *Cyp9J32* in *A. aegypti*, and *Cyp6P12* in *A. albopictus* were assessed using qPCR. *A. aegypti* adults G0 were screened using real time melting curve qPCR analyses to genotype the F1534C, V1016I and V410L *Aedes kdr* mutations. Overall, *A. aegypti* is the predominant *Aedes* species, but analyses revealed that both *A. albopictus* and *A. aegypti* coexist in all the prospected neighbourhoods of Douala. High level of resistance was observed to three pyrethroids tested in both *Aedes* species. In *A. aegypti* a lower mortality rate was reported to permethrin (5.83%) and a higher mortality rate to deltamethrin (63.74%). Meanwhile, for *A. albopictus*, lower (6.72%) and higher (84.11%) mortality rates were reported to deltamethrin. Similar analysis with bendiocarb, revealed for *A. aegypti* a loss of susceptibility. However, in *A. albopictus* samples, analyses revealed a susceptibility in Logbessou, and confirmed resistance in Kotto (59.78%). A partial recovery of mortality was found to insecticides after pre-exposure to PBO. *Cyp6P12* was found significantly overexpressed in *A. albopictus* permethrin resistant and *Cyp9M6F88/87* for *A. aegypti* deltamethrin resistant. F1534C, V1016I and V410L mutations were detected in *A. aegypti* from different neighbourhoods and by considering the combination of these three *kdr* 14 genotypes were found. These findings provide relevant information which should be capitalised in the implementation of arbovirus vector control strategies and insecticide resistance management.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Aedes-borne viral diseases such as dengue, chikungunya, yellow fever and Zika are increasingly reported in Cameroon. Recently it was demonstrated that 13% of acute febrile patients consulting hospitals in Douala, Cameroon are due to dengue. During this study the co-circulation of three dengue serotypes, DENV-1, DENV-2, and DENV-3, were reported [1]. The viruses responsible for these diseases are transmitted by the infected bite of mosquitoes of the genus *Aedes*, whose major vectors are *Aedes aegypti* Linnaeus, 1762 and *Aedes albopictus* (Skuse), 1894. *Aedes aegypti* and *A. albopictus* are from different origins. *A. aegypti* originates from Africa [2] and is found throughout the tropics, while *A. albopictus* is native to Asia [3], but has invaded all the continents including Africa [4]. Both *Aedes* species are present in Cameroon with discrepancy in distribution. *A. aegypti* is distributed across the country while *A. albopictus* is restricted to the southern part of the country in latitude 6°N where it tends to supplant the native species *A. aegypti* [5–7]. In cities where both species are found, *A. aegypti* is found most prevalent in downtown neighbourhoods with high building density while *A. albopictus* is mostly found in peri-urban neighbourhoods surrounded by vegetation [5,8–10].

In absence of effective vaccine and specific treatment against this arboviruses, dengue fever prevention relies on vector control through reduction of larval sources and environment management, but during an epidemic, space spraying of insecticides is generally used to target adult mosquitoes [11]. Unfortunately, vector control is counteracted by the emergence and the continued development of resistance to all classes of insecticides used in public health [12].

Two main mechanisms are implicated in *Aedes* mosquitoes' resistance to insecticides: the metabolic resistance and the target site resistance. Metabolic resistance is mediated by upregulation of detoxification enzymes such as the monooxygenases (cytochrome P450s), glutathione S-transferases (GSTs) and carboxylesterases (COEs) [13]. Many genes of the P450 family, especially from the CYP9 and CYP6 subfamilies (*CYP9J28*, *CYP9J10*, *CYP9J26*, *CYP6BB2* and *CYP6P12*) have been associated with resistance to pyrethroids [12,14–18]. Target site resistance is caused by mutations in target genes such as the acetylcholinesterase (*Ace-1*), the GABA receptor and the voltage-gated sodium channel (VGSC) which causes knockdown resistance (*kdr*). The *kdr* is one of the main target site resistance mechanisms known as involved in the resistance for both pyrethroids and dichlorodiphenyltrichloroethane (DDT) insecticides [19–21]. In *A. aegypti*, 11 *kdr* mutations at 9 different codons positions in the VGSC domains I-IV have been reported [12,22]. Among them F1534C, V410L and V1016G, have already been identified in Cameroon [23–25]. Till date, only four VGSC mutations have been detected in *A. albopictus* affecting two codons (1532 and 1534). This study presents the spatial distribution and the insecticide susceptibility profile of *A. aegypti* and *A. albopictus* and the potential mechanisms implicated in the resistance of these arbovirus vectors in the city of Douala.

Material and methods

Mosquito collection and rearing

Aedes mosquitoes were sampled as larvae or pupae in August 2020 (small rainy season) in eight neighbourhoods in Douala (Fig 1). Based on previous studies in Cameroon showing that *A. albopictus* is prevalent in neighbourhoods located in suburban areas of the city while *A. aegypti* is predominant in the downtown [6,9], we selected four urban neighbourhoods: central Akwa (N 04°02.934'; E 009°41.475'), Bépanda (N 04°03.303'; E 009°43.272'), Brazzaville (N 04°

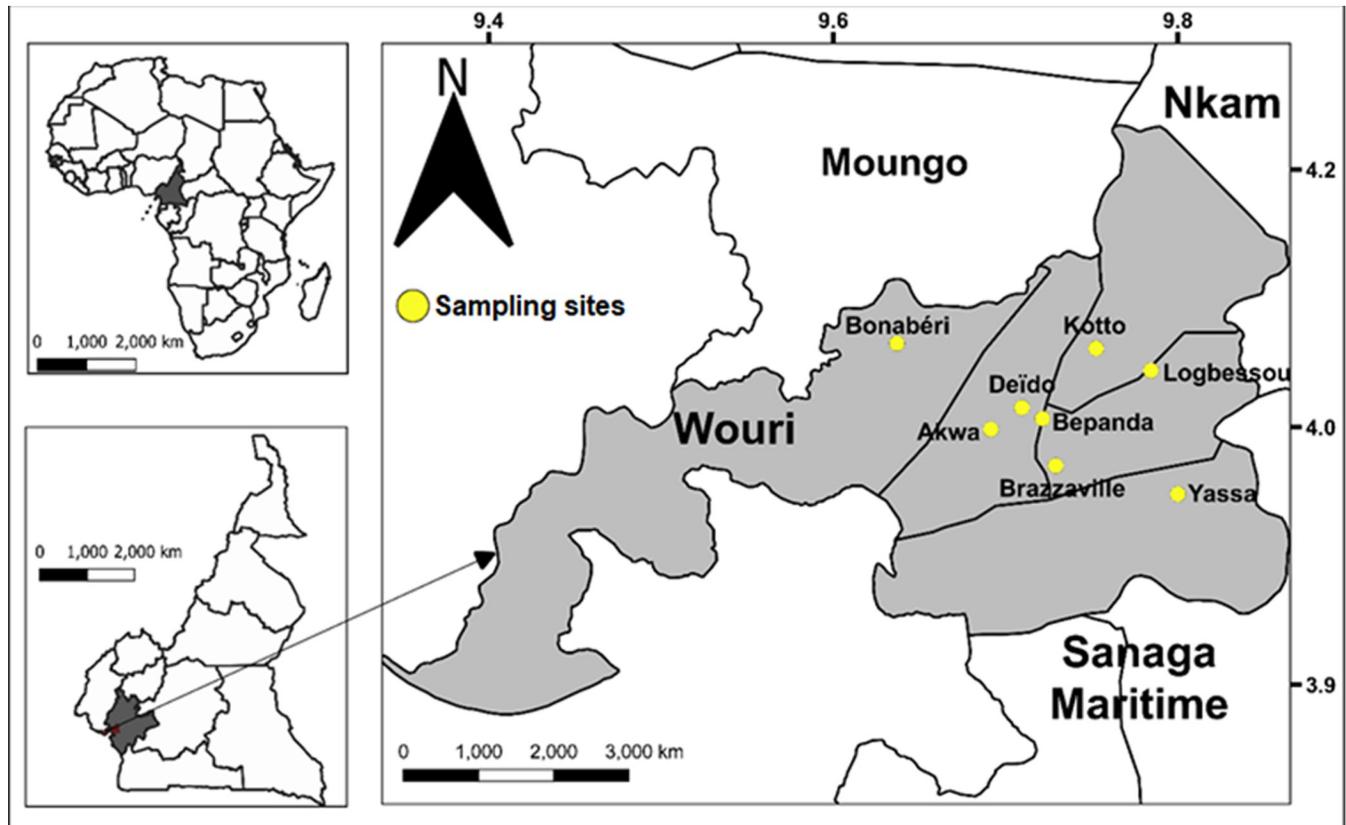


Fig 1. Map of study site. QGIS version 3.14.16, was used to generate the map using open access share files (<https://gadm.org/>).

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1.6570°; E 009° 43.7310') and Deïdo (N 04° 03.690'; E 009° 42.560') and four peri-urban neighbourhoods: Bonabéri (N 04° 04.214'; E 009° 41.155'), Kotto (N 04° 05.753'; E 009° 45.141'), Logbessou (N 04° 04.983'; E 009° 47.050') and Yassa (N 04° 00.661'; E 009° 47.983'). Immature stages (field generation, G0) were collected from different breeding sites: domestic (e.g. tanks), peri-domestic (e.g. used tires, discarded tanks), and natural (e.g. tree holes). In each location, larvae or pupae from at least 20 positive larval breeding places were collected, stored in plastic boxes, and transferred to the insectary, pooled according to the neighbourhoods, and reared to adult stages. Adult mosquitoes were morphologically identified using taxonomic keys [26], numbered, pooled in a breeding cage according to species and location and further reared in controlled conditions ($27 \pm 2^\circ\text{C}$; relative humidity $80 \pm 10\%$) until generation 1 (G1) for adult bioassays. The comparison between the prevalence of *A. aegypti* and *A. albopictus* in each neighbourhood was performed using chi-square tests.

Insecticide resistance bioassays

Bioassays were performed according to WHO protocol using 2–5 days old G1 generation. Four replicates of 20–25 females per tube were exposed to 0.03% deltamethrin, 0.05% alphacypermethrin, 0.1% bendiocarb and 0.75% permethrin for 1 hour. Mortality was recorded 24 hours later and mosquitoes alive or dead after exposure were stored in RNA later or silica gel, respectively. The resistance status was defined as follows: susceptible (mortality rate between 98–100%), probable resistance (mortality rate between 90–97%), and resistant (mortality rate inferior to 90%) [27].

Adult synergist assay with PBO

To evaluate the potential role of oxidase specific metabolic resistance mechanisms, synergist assays with 4% piperonyl butoxide (PBO) were performed. 2-5-days-old adults were pre-exposed for one hour to PBO-impregnated papers and after that immediately exposed to the selected insecticide. Mortality was scored 24 hrs later and compared to the results obtained with each insecticide without synergist according to the WHO standards [27]. The comparison of mortality rates after pre-exposure of mosquitoes to synergist and without pre-exposure to synergist was done using Chi-square test. The difference was statistically different when *P*-value was inferior to 0.05.

Knockdown resistance (*kdr*) genotyping

Three *kdr* mutations (V1016I, V410L and F1534C) were genotyped using around 30 samples per neighbourhood. Genomic DNA extracted from 30 individual mosquitoes per populations [28] were used for this experiment. Genotyping of the V1016I, V410L and F1534C mutations was performed by the real-time quantitative PCR using protocol published by Saavedra-Rodriguez et al. [29,30]. Each PCR reaction was performed in a 21.5 μ L mixture containing 2 μ L of DNA sample, 10 μ L of SYBR[®] Green (SuperMix), 1.25 μ L of each primer and 5.75 μ L of ddH₂O. The thermocycle parameters were: 95°C for 3 min, followed by 40 cycles of 95°C for 20 s, 60°C for 1min and 72°C for 30 s and then a final step of 72°C for 5 mins, 95°C for 1 min, 55°C for 30 s and 95°C for 30 s.

Expression of detoxification Genes

RNA extraction and cDNA synthesis. For this experiment three groups of mosquitoes were used: the unexposed (control), the exposed (resistant) and the susceptible (laboratory susceptible strains). For each group three replicates of 10 mosquitoes per species were set up. RNA was extracted using the PicoPure RNA Isolation Kit (Arcturus[®] PicoPure RNA Extraction Kit Life Technologies, California, USA), following the manufacturer's recommendations. Quality and quantity of RNA obtained were assessed using a "NanoDrop Lite" spectrophotometer (Thermo Scientific Inc., Wilmington, USA) and was stored at -80°C until further use.

Extracted RNA was used to synthesise complementary DNA (cDNA) using the Superscript III kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and the resulting cDNA was purified using a QIAquick spin column (QIAquick PCR Purification Kit, Qiagen) and diluted 2-fold to accommodate the volumes of the reaction.

Quantitative-reverse transcriptase PCR. Expression profiles of genes previously associated with *A. aegypti* or *A. albopictus* pyrethroid resistance [12,31] were assessed using quantitative reverse transcription PCR (qRT-PCR), in relation to two susceptible strains, Benin strain for *A. aegypti* [32,33] and Vector Control Research Unit (VCRU) strain for *A. albopictus* [34,35]. Standard curve analyses were performed for each primer pair to check the specificity and efficiency of amplifications. Four cytochrome P450 candidate genes were chosen for analysis in *A. aegypti* (*Cyp9M6F88/87*, *Cyp9J28a*, *Cyp9J10* and *Cyp9J32*) and only one in *A. albopictus* (*Cyp6P12*). The reactions were performed in a mixture of 20 μ L with 10 μ L sybrGreen (Applied Biosystems, Texas, USA), 0.6 μ L of each primer (10 μ M), 7.8 μ L of ddH₂O and 1 μ L of CDNA, under the following conditions: 95°C for 3 min, followed by 40 cycles of 95°C for 10 s and 60°C for 10 s. The relative expression level and fold change (FC) of each candidate gene compared to susceptible strains were calculated using the 2- $\Delta\Delta$ CT method integrating the efficiency of PCR [36] after normalization with housekeeping genes: *Aaeg60sL8*, *RPS3*, *RSP7* and *qTubulin* (S1 Table). All the primer sequences are presented in Table 1 respectively for *A.*

Table 1. Primer sequences used to evaluate the level of expression of metabolic resistance genes by RT-qPCR in *Aedes albopictus* and *Aedes aegypti*.

Genes	Forward primer	Reverse primer
<i>Aedes albopictus</i>		
<i>Cyp6P12</i>	CGTGCGCTTTTGGGATTGAG	ATCGTCCGTGCCAAATCCTT
<i>RSP7</i>	AAGGTCGACACCTTCACGTC	CGCGCGCTCACTTATTAGAT
<i>qTubulin</i>	CCGCACTCGAGAAGGATTAC	GTGGTTCGGTTTGACTTCGT
<i>Aedes aegypti</i>		
<i>Cyp9J10</i>	ATCGGTGTTGGTGAAAGTTCTGT	CATGTCGTTGCGCATTATCCC
<i>Cyp9J28</i>	CCACTGACGTACGATGCGA	GCCGATCAGTGGACGGAGC
<i>Cyp9M6</i>	TCGGTGCACAATCCAACAAC	GTGGGTACGACCAACGAAA
<i>Cyp9J32</i>	CGGTCCGCTTATGACGAAGAG	TTGTTCGCTCCGAAGAGTGG
<i>RPS3</i>	AGCGTGCCAAGTCGATGAA	GTGCCGTGTCGACGTACT
<i>Ae60sL8</i>	CTGAAGGGAACCGTCAAGCAA	TCGGCGCAATGAACAAC

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albopictus and *A. aegypti*. The Mx Pro software integrated into the Agilent brand TaqMan machine was used.

Results

Relative abundance and spatial distribution of *A. aegypti* and *A. albopictus* in Douala

A total of 13,927 specimens of *Aedes* spp. comprising 8,564 (61.49%) individuals belonging to *A. aegypti* species and 5,363 (38.51%) to *A. albopictus* species (Table 2) were collected during this study. Analysis revealed that both *Aedes* species coexist in all the prospected neighbourhoods in Douala but overall, *A. aegypti* is the predominant species (Fig 2). According to the environment (downtown vs suburban), *A. aegypti* is more predominant in all the neighbourhoods located in the downtown environment (Akwa, Brazzaville, Bépanda and Deïdo) while

Table 2. Abundance of *A. aegypti* and *A. albopictus* from different locations in Douala.

Environment	Location	Breeding sites	Number of prospected breeding sites	Number of positive breeding sites	<i>A. aegypti</i>	<i>A. albopictus</i>	<i>P</i> -value	
Downtown	Akwa	Used tires	38	25	778	230	< 0.001	
		Discarded tanks	3	2				
		Car wrecks	1	1				
	Bépanda	Used tires	60	37	1592	182	< 0.001	
		Car wrecks	1	1				
	Brazzaville	Used tires	104	56	1865	51	< 0.001	
	Deïdo	Used tires	38	22	973	465	< 0.001	
		Factory molds	50	30				
	Total 1			295	174	5208	928	< 0.001
	Suburban	Bonabéri	Used tires	69	58	1681	503	< 0.001
Discarded tanks			7	7				
Kotto		Used tires	84	37	291	1471	< 0.001	
		Discarded tanks	2	1				
		Miscellaneous	1	1				
Logbessou		Used tires	141	82	516	1378	< 0.001	
Yassa		Used tires	252	129	868	1083	< 0.001	
Total 2			556	315	3356	4435	< 0.001	
Total 1+Total 2			851	489	8564	5363	< 0.001	

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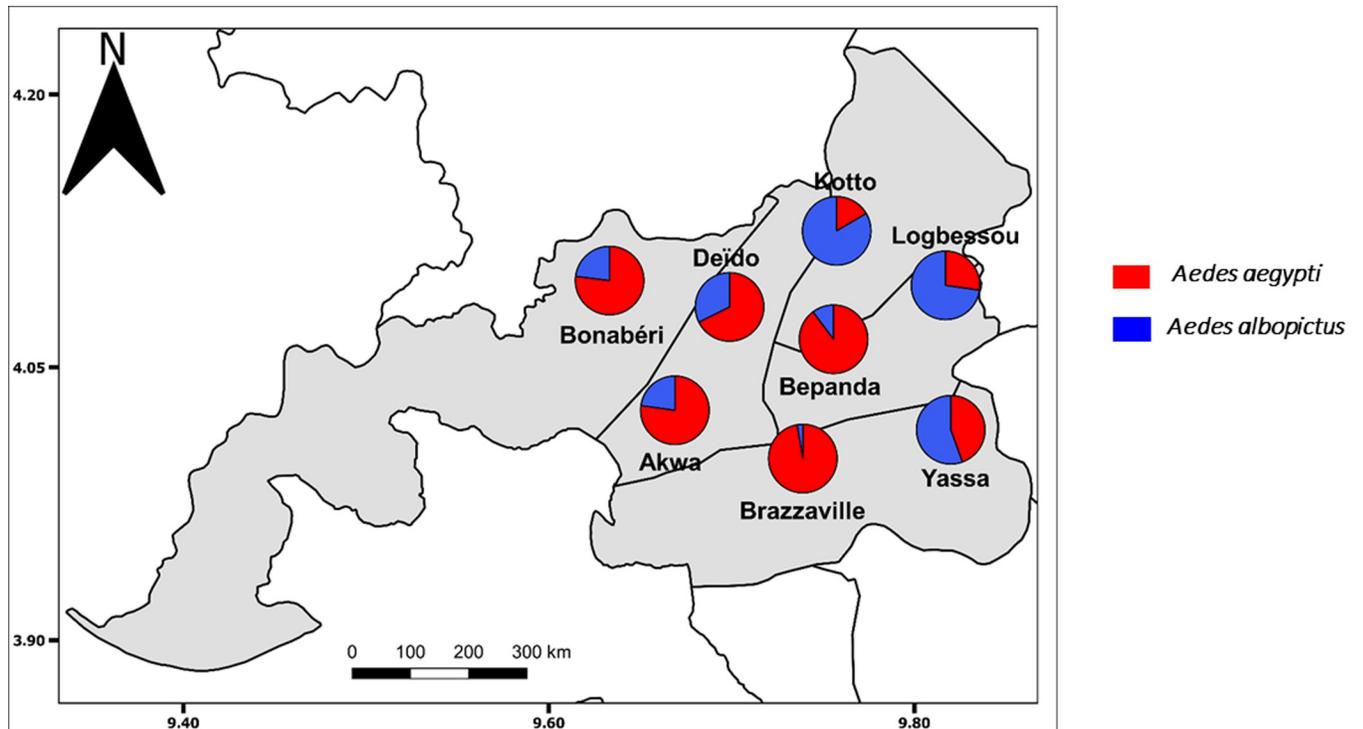


Fig 2. Geographical distribution of *A. aegypti* and *A. albopictus* in the city of Douala, Cameroon.

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A. albopictus is rather predominant in suburban neighbourhoods (Logbessou, Kotto and Yassa) except in Bonabéri where *A. aegypti* largely dominated over *A. albopictus* ($\chi^2 = 2758.8$; $P < 0.001$).

Insecticide resistance profile of *A. aegypti* and *A. albopictus* in Douala

The results of adult bioassays showed a level of susceptibility varying according to the neighbourhood and the insecticide used. Three pyrethroids (0.03% deltamethrin, 0.75% permethrin and 0.05% alphacypermethrin) and one carbamate (0.1% bendiocarb) were used for adult bioassays.

Insecticide resistance profile of *A. aegypti* in Douala. All populations tested were resistant to three pyrethroids used with mortality rates varying from 23.30% (Brazzaville) to 63.74% (Yassa) to deltamethrin, from 6.57% (Brazzaville) to 55.45% (Yassa) to alphacypermethrin and from 5.83% (Brazzaville) to 60.16% (Yassa) to permethrin. With respect to bendiocarb, we observed resistance in Akwa and Yassa with mortality rates of 70.62% and 85.89% respectively. Probable resistance was observed in Brazzaville, Bonabéri and Logbessou with mortality rate between 91.79 and 94.91% (Fig 3).

Insecticide resistance profile of *A. albopictus* in Douala. All populations tested were resistant to all pyrethroids used with mortality rates varying from 6.72% (Deïdo) to 84.11% (Logbessou) to deltamethrin, from 24.35% (Kotto) to 71.21% (Logbessou) to alphacypermethrin, and from 59.26% (Deïdo) to 81.15% to permethrin. To bendiocarb, we observed susceptibility in Logbessou, probable resistance in Bonabéri and Yassa (mortality rate around 96%), confirmed resistance in Deïdo and Kotto (mortality rates of 74.17 and 59.78% respectively) (Fig 4).

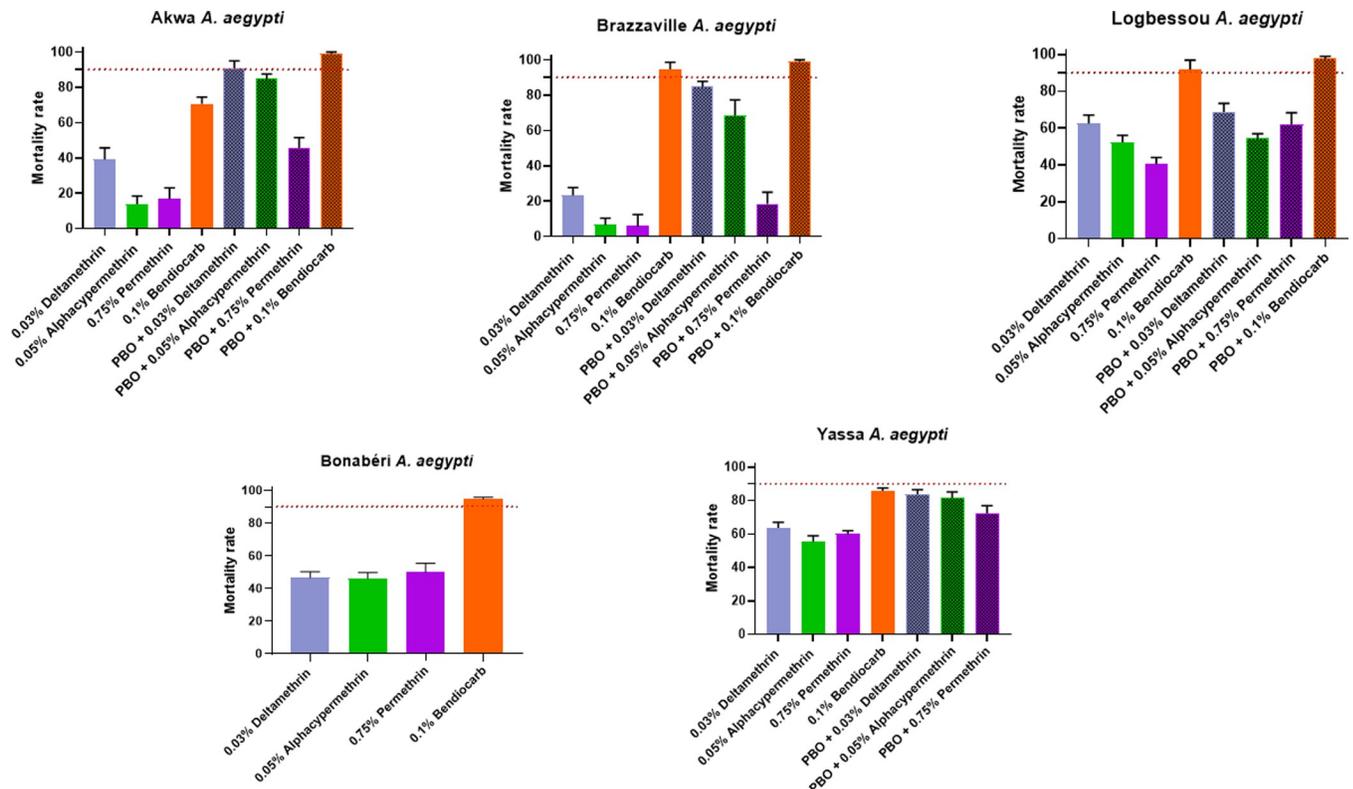


Fig 3. Mortality rates of adult *Aedes aegypti* from five locations (per environment) in Douala when exposed to insecticides alone or with 1 h pre-exposure to synergist. Error bars represent standard error of the mean. PBO Piperonyl butoxide.

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Tests with synergist PBO. To evaluate if the resistance observed in some populations was due by the Cyt-P450 genes, mosquitoes were pre-exposed to synergist PBO. The analyses revealed a significant recovery of susceptibility to insecticide with pre-exposure to PBO in most populations tested for both species. This was the case of *A. aegypti* from Logbessou where a recovery of susceptibility was reported to deltamethrin ($84.11 \pm 3.09\%$ of mortality without PBO pre-exposure vs $97.28 \pm 0.90\%$ of mortality after PBO pre-exposure, $P < 0.05$), permethrin ($81.15 \pm 3.97\%$ of mortality without PBO pre-exposure vs $99.10 \pm 0.89\%$ of mortality after PBO pre-exposure, $P < 0.05$), and alphacypermethrin ($71.21 \pm 8.69\%$ of mortality without PBO pre-exposure vs $95.94 \pm 1.73\%$ of mortality after PBO pre-exposure, $P < 0.05$).

Expression of detoxification Genes. The qPCR analyses revealed that the only gene (*Cyp6P12*) whose expression was assessed in *A. albopictus* populations was found to be significantly overexpressed in the permethrin resistant Deïdo samples compared to susceptible VCRU lab strain ($FC = 5.54 \pm 0.73$, $P = 0.001$); whereas in the deltamethrin-resistant Deïdo samples and the alphacypermethrin-resistant Yassa samples, the expression was not significantly different (Fig 5).

Two among the four Cyt-P450 genes assessed in *A. aegypti* were significantly overexpressed in field populations compared to susceptible Benin lab stain (Figs 6 and 7). *Cyp9M6F88/87* was overexpressed in Logbessou samples resistant to deltamethrin ($FC = 5.49 \pm 1.64$, $P = 0.003$) and in Brazzaville samples resistant to permethrin ($FC = 2.54 \pm 0.90$, $P = 0.016$); while *Cyp9J10* was overexpressed in Logbessou samples and resistant to deltamethrin ($FC = 3.16 \pm 0.40$, $P = 0.013$).

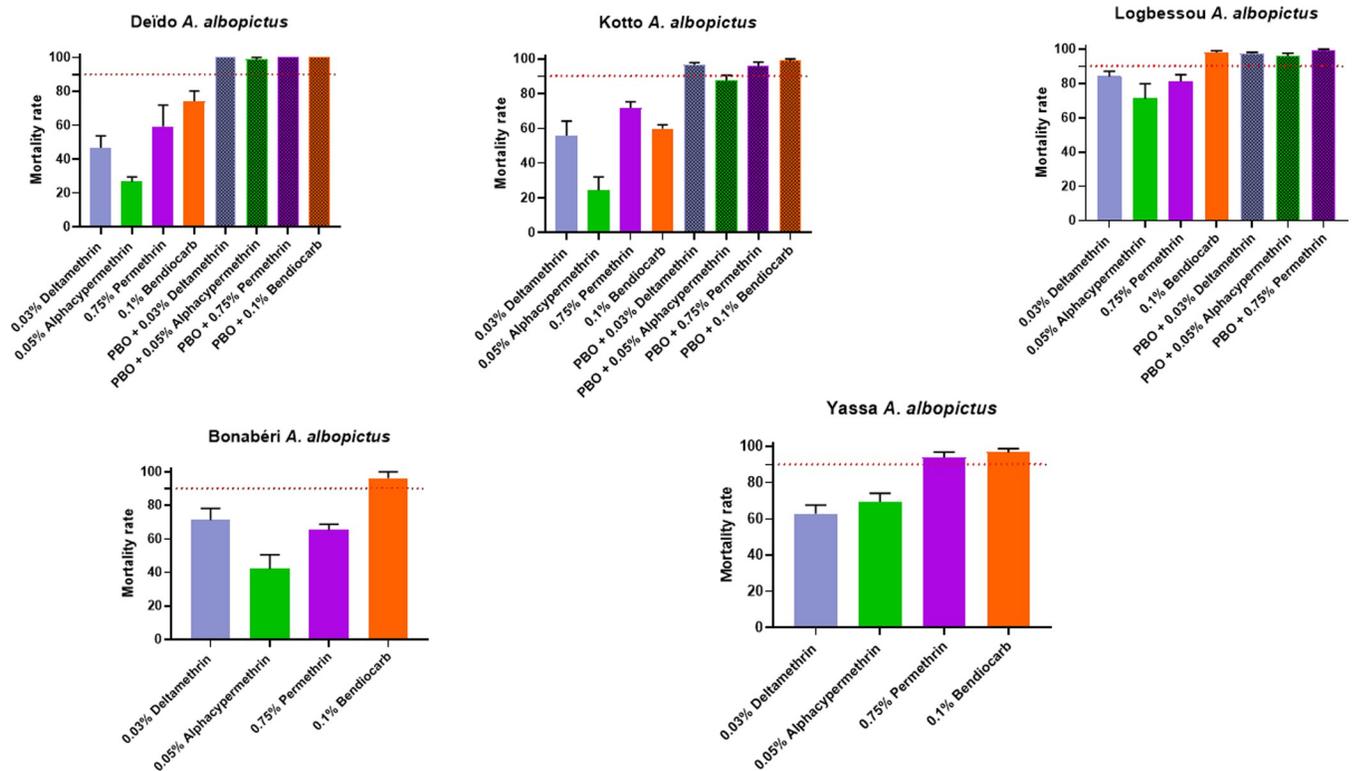


Fig 4. Mortality rates of adult *Aedes albopictus* from five locations (per environment) in Douala when exposed to insecticides alone or with 1 h pre-exposure to synergist. Error bars represent standard error of the mean. PBO Piperonyl butoxide.

<https://doi.org/10.1371/journal.pone.0278779.g004>

Knockdown resistance (kdr) genotyping. Three *kdr* mutations were genotyped F1534C, V1016I and V410L in eight locations of Douala (Table 3).

Concerning V1016I *kdr* genotyping, a total of 230 samples were examined. Among these, 131 (56.96%) were homozygote wild type (1016V/V), 76 (33.04%) were heterozygote (1016V/I) and 23 (10%) were homozygote mutant (1016I/I). Overall, allelic frequency of homozygote wild type was 73.48% while for homozygote mutant was 26.52% (S1 Fig).

For V410L *kdr* genotyping, we examined around 226 samples of which 125 (55.31%) were homozygote wild type (410V/V), 78 (34.51%) were heterozygote (410V/I) and 23 (10.18%) were homozygote mutant (410I/I). Overall, allelic frequency of homozygote wild type was 72.57% while for homozygote mutant was 27.43% (S2 Fig).

A total of 238 specimens were examined for F1534C genotyping. Overall allelic frequencies were 12.61% for homozygote wild type and 87.39% for homozygote mutant (S3 Fig). Analysis revealed that 16 (6.72%) were homozygote wild type (1534F/F), 28 (11.77%) were heterozygote (1534F/C) and 194 (81.51%) were homozygote mutant (1534C/C).

By considering the three *kdr* together (1016+410+1534), 225 samples of *A. aegypti* were analyzed and we found 14 genotypes: VV/VV/FF, VV/VV/CC, VV/VV/FC, VI/VV/CC, VV/VL/CC, VV/VL/FC, VV/LL/FC, VV/LL/CC, VI/VV/FC, VI/VL/CC, VI/VL/FC, II/LL/CC, II/VL/CC and VI/LL/CC (Fig 8). The frequency of each genotype varies according to the location. The only one susceptible genotype VV/VV/FF was present in five locations of the eight locations tested. The high frequency of this genotype was 37% (Yassa samples) followed by 7% (Kotto samples). The most predominant genotype VV/VV/CC was found in all the eight locations analyzed with frequency between 15 and 69% in Deido and Brazzaville respectively. Overall, the

Expression of *CY6P12* gene in *A. albopictus*

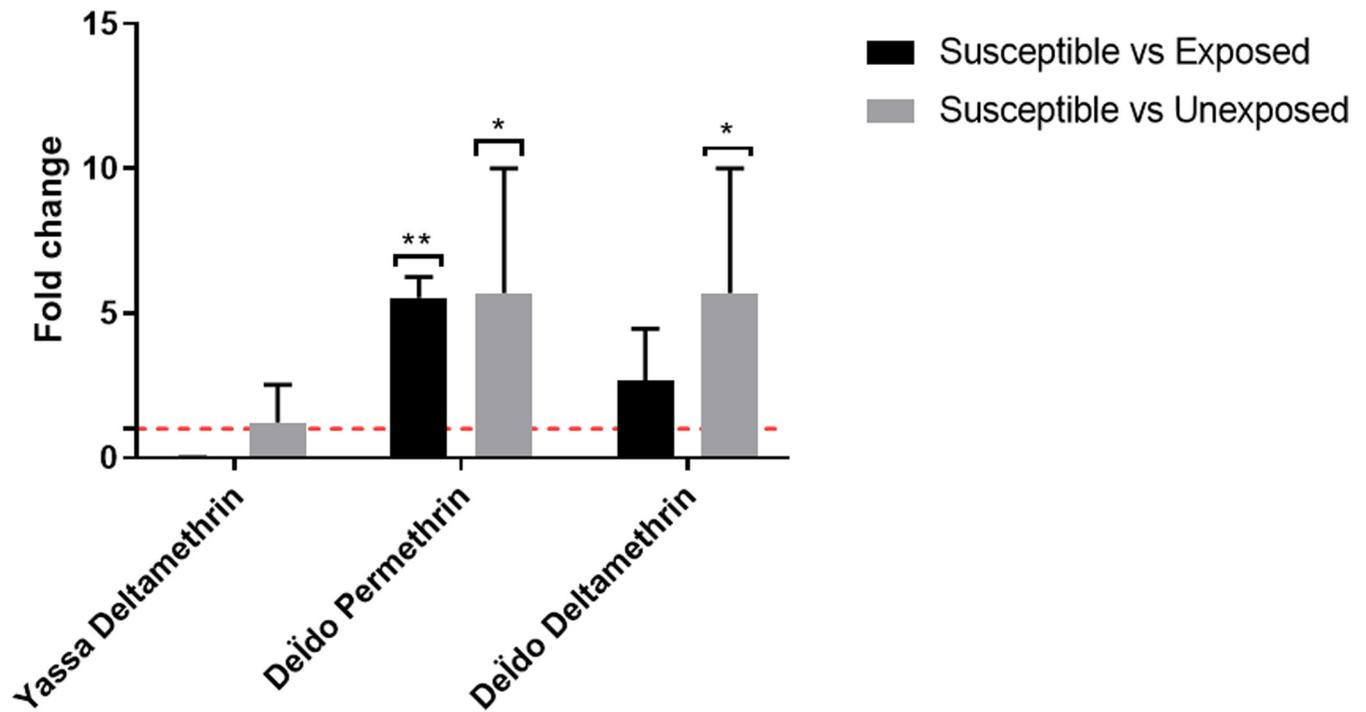


Fig 5. Expression of the *CYP6P12* gene in *A. albopictus* samples from Douala city.

<https://doi.org/10.1371/journal.pone.0278779.g005>

frequency of this genotype was 40,88% within the Douala *A. aegypti* population. The second dominant genotype was VI/VL/CC present in all the eight locations with a frequency between 3% and 38%. The frequency of this genotype in the population was 25.33%. We noticed that some genotypes were present in only five locations (VV/VV/FC and II/LL/CC), four locations (VI/VV/CC and VI/VL/FC), three locations (VV/VL/CC), two locations (II/VL/CC) or in only one location (VV/VL/FC, VV/LL/FC, VV/LL/CC, VI/VV/FC and VI/LL/CC).

Discussion

This study aimed to assess the spatial distribution, the insecticide resistance profile of *A. aegypti* and *A. albopictus* in Douala and to explore the potential resistance mechanisms involved.

Spatial distribution of *A. aegypti* and *A. albopictus* in Douala

Results showed that both species are present in all prospected neighbourhoods. *Aedes aegypti* was the predominant species in all neighbourhoods located in downtown while *A. albopictus* was more predominant in suburban localities. Previous studies reported the same observation in the city of Douala [5,6].

The predominance of *A. aegypti* in the downtown is consistent with previous works on the spatial distribution of *A. aegypti* and *A. albopictus* in other cities in Central Africa [8,10], in Asia [37] and in South America [38]. In these localities, it was clearly demonstrated that *A. aegypti* preferentially colonises man-made containers located in areas with high building density [6,37,39], whereas *A. albopictus* preferentially colonised breeding sites surrounded by vegetation [5,6,8]. In this study the predominant breeding sites were used tyres, which are described in

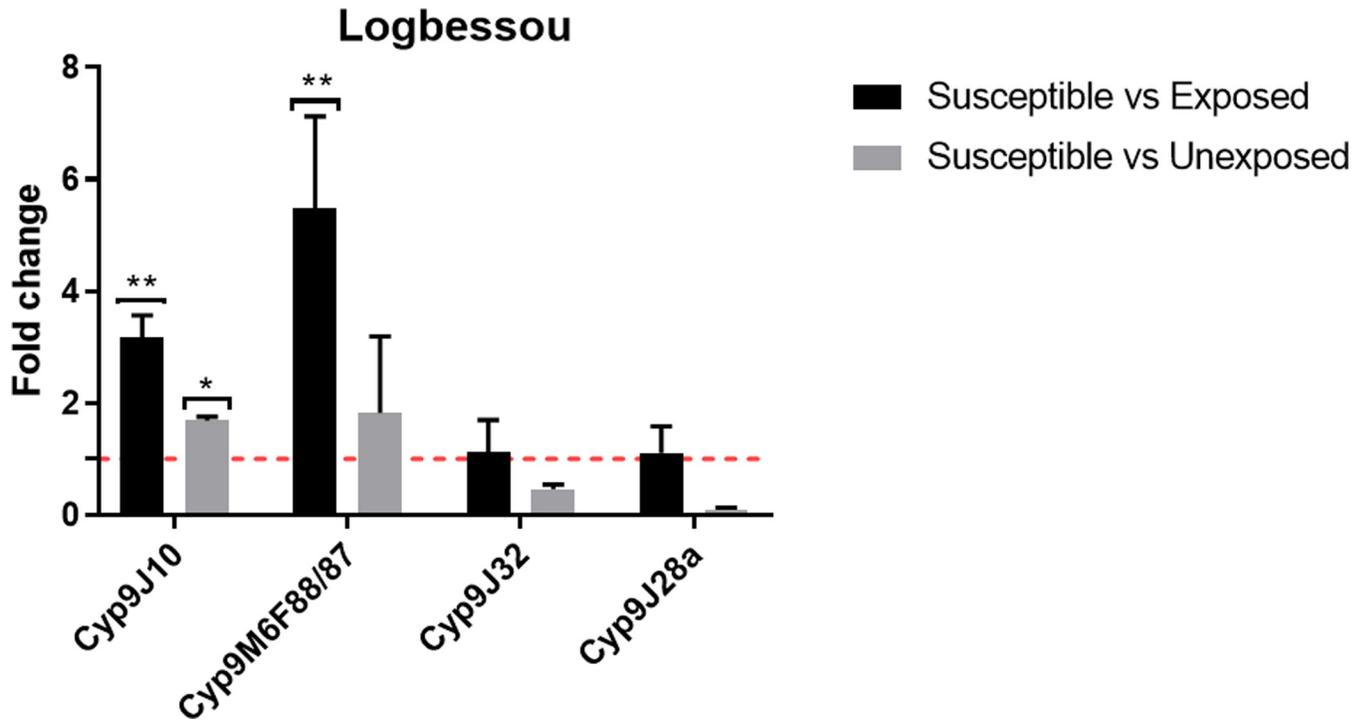


Fig 6. Expression of the *CYP9J10*, *CYP9M6F88/87*, *CYP9J32* and *CYP9J28a* genes in *A. aegypti* samples from Logbessou, Douala city.

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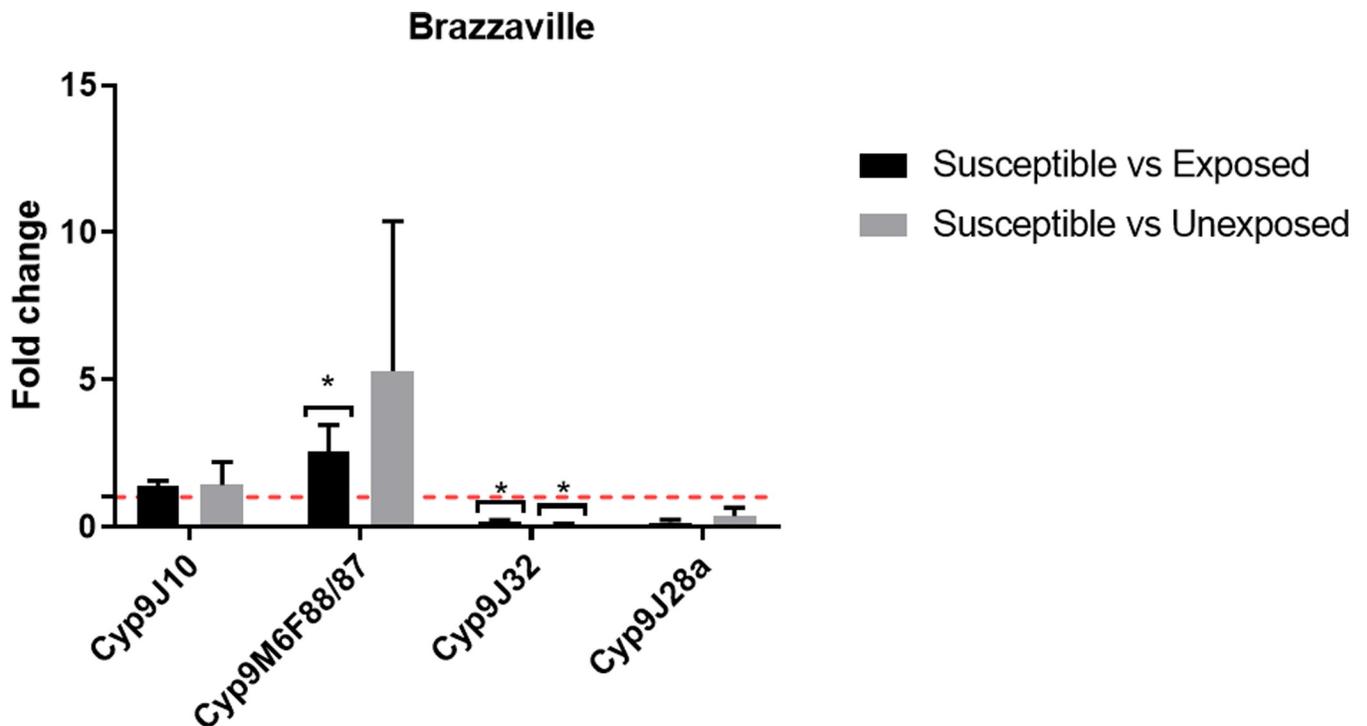


Fig 7. Expression of the *CYP9J10*, *CYP9M6F88/87*, *CYP9J32* and *CYP9J28a* genes in *A. aegypti* samples from Brazzaville, Douala city.

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Table 3. Genotypes of *Aedes aegypti* populations from Douala, Cameroon considering V1016I, V410L *kdr* and F1534C mutation in the VGSC.

qPCR results	VV/VV/FF	VV/VV/CC	VV/VV/FC	VI/VV/CC	VV/VL/CC	VV/VL/FC	VV/LL/FC	VV/LL/CC	VI/VV/FC	VI/VL/CC	VI/VL/FC	II/LL/CC	II/VL/CC	VI/LL/CC	n
Location															
Yassa	0.37	0.4	0.17	0	0	0	0	0	0	0.03	0.03	0	0	0	30
Bonaberi	0.04	0.31	0.1	0.04	0.03	0.03	0	0	0	0.38	0	0.07	0	0	29
Bepanda	0	0.23	0	0	0	0	0	0	0	0.33	0.07	0.34	0.03	0	30
Logbessou	0.04	0.57	0.13	0.03	0	0	0	0	0	0.23	0	0	0	0	30
Deïdo	0	0.15	0	0.11	0.15	0	0.04	0.07	0	0.3	0	0.04	0.07	0.07	27
Kotto	0.07	0.44	0	0.04	0	0	0	0	0	0.34	0.04	0.07	0	0	27
Brazzaville	0	0.69	0.08	0	0	0	0	0	0	0.19	0.04	0	0	0	26
Akwa	0.04	0.5	0.08	0	0.04	0	0	0	0.03	0.23	0	0.08	0	0	26

n: Number of samples tested; qPCR results (1016+410+1534); V: Valine; I: Isoleucine; L: Leucine; F: Phenylalanine; C: Cysteine.

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previous studies as preferential breeding sites for *Aedes* [8] because they offer not only very good shelter from predators but also from heat. The suburban neighbourhoods (Kotto, Logbessou, Yassa) which have a high vegetation cover compared to the downtown neighbourhoods (Deïdo, Akwa, Brazzaville and Bépanda) therefore offer a more favourable environment for the development of *A. albopictus*. This is consistent with previous studies conducted in some suburban neighbourhoods of Douala showing that *A. albopictus* is the predominant species in this environment [40]. Overall, analyses revealed that *A. aegypti* is the predominant species in the city of Douala, which corroborates previous observations [5,6]. This suggests that the environmental factors in Douala are more favourable to the development of this species in contrast to what is generally observed in localities in the southern part of Cameroon located below 6° N [5].

Insecticide resistance profile of *A. aegypti* and *A. albopictus*

This study revealed that both species *A. aegypti* and *A. albopictus* are resistant to all insecticides tested (deltamethrin, alphacypermethrin, permethrin and bendiocarb). Indeed, resistance to

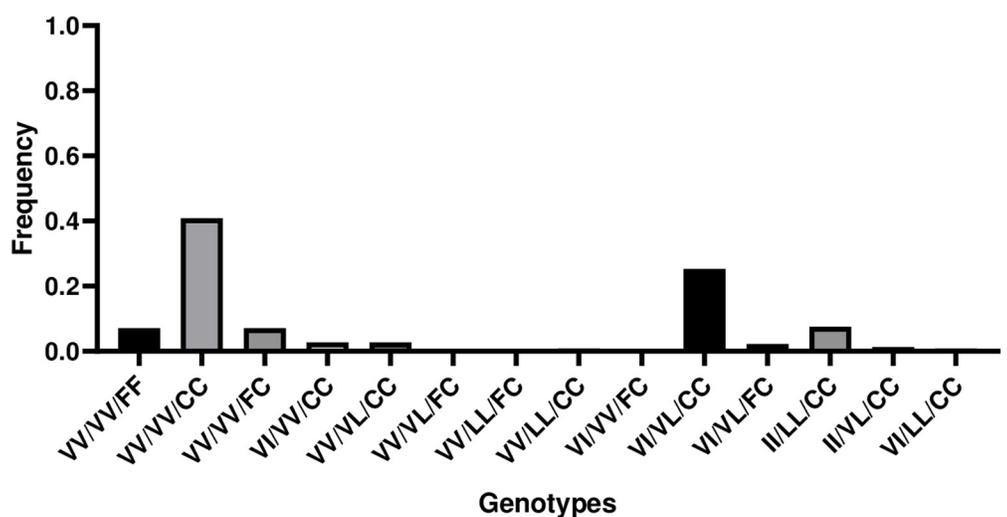


Fig 8. Frequency of *kdr* genotypes in *Aedes aegypti* mosquito from Douala, Cameroon considering F1534C, V1016I and V410L *kdr* mutation in the VGSC.

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permethrin 0.75% and deltamethrin 0.03% had already been reported in *A. aegypti* in Douala [23], in other cities in Cameroon [9,23], and other regions of the world such as Saudi Arabia [41] and Burkina Faso [42]. The resistance to 0.03% deltamethrin and 0.75% permethrin observed in *A. albopictus* corroborates with those previously obtained in Congo [43], in Vietnam, in Singapore and in Italy [44] for permethrin and in Yaoundé for deltamethrin [9].

Globally, *A. aegypti* is more resistant to pyrethroids than *A. albopictus*. Similar results were obtained in other parts of the world including Malaysia [45]. However, these observations are different from those reported in the city of Yaoundé (Cameroon), indicating that the level of resistance observed in *A. albopictus* to pyrethroids was higher than in *A. aegypti* [9]. The resistance of *A. aegypti* and *A. albopictus* to pyrethroids could pose a serious threat to vector control programs, because pyrethroids are the main class of insecticide recommended for the control of adult *Aedes* mosquitoes notably in case of outbreaks [11].

A loss of susceptibility to bendiocarb was observed for both *Aedes* species. Similar patterns have been observed previously in Saudi Arabia [41], in Burkina Faso [42], Pakistan [46] and Malaysia [45].

Resistance or loss of susceptibility observed to these insecticides (deltamethrin, permethrin and bendiocarb) in both *Aedes* species is difficult to explain because in Central Africa, specific insecticide treatments targeting *Aedes* spp. are currently very limited or non-existent [9,47]. This observation raises the question of the origin of the selection pressure by these species. As suggested previously [9,47], domestic use of insecticides through Indoor Residual Spraying (IRS) and impregnating bed nets, mass distribution of long-lasting insecticidal nets (LLINs) by national malaria control programme, and agriculture use could be the main source of resistance selection in *Aedes* vectors in Central Africa. Indeed, the use of pesticides in agriculture for the protection of market gardening could also promoted the emergence of resistance in mosquitoes by contamination of breeding sites and resting places of mosquitoes.

Resistance mechanism involved

Synergist assay with pre-exposure to PBO showed a partial or full recovery of susceptibility to all insecticides used in *A. aegypti* and *A. albopictus*. These observations are consistent with previous studies in Africa [9,18,23,43,48] and abroad [14,15]. This result indicates that the cytochrome P450 monooxygenases are playing the main role in the resistance of deltamethrin, alphacypermethrin, permethrin and bendiocarb. This result is supported by the overexpression of some genes analysed such as *CYP6P12* in Deïdo in *A. albopictus* samples resistant to permethrin or *Cyp9M6F88/87* in Logbessou and Brazzaville in *A. aegypti* samples resistant to deltamethrin and permethrin respectively. The *CYP6* and *CYP9* sub-families, were previously found to be associate with resistance to pyrethroids [14,15].

In this study three *kdr* mutations F1534C, V1016I and V410L known as involved in pyrethroids resistance in *Aedes* mosquito are reported. These mutations have already been reported in Cameroon [23–25] and other countries in Africa [18,42,49–52] and abroad [22].

The F1534C mutation is common in *A. aegypti* and has a cosmopolitan distribution [12]. This mutation has been reported in West Africa including Ghana [21] and Burkina Faso [18,42]. Although not previously reported in Central Africa, previous work of Yougang *et al.* reported for the first time the existence of this mutation in *A. aegypti* in two cities in Cameroon [23]. The results of this work confirmed the presence of this *kdr* mutation in the city of Douala with a very high allelic frequency (87.39%). This high allelic frequency is similar to previous one reported in Douala [24]. The occurrence of these mutations is likely to impact the effectiveness of pyrethroid-based control measures against *A. aegypti* currently implemented in some African countries [51]. The combination of two of these mutations or all three mutations

contribute strongly to increase resistance in *A. aegypti*. For example, V410L, alone confers low levels of resistance but co-evolved with V1016I or F1534C yielding higher levels of resistance [18,22,51]. Considering the tree *kdr* mutations together 14 genotypes were found, among them two were the most abundant: VV + VV + CC and VI + VL + CC (1016+410+1534). Similar observation was reported in Brazil [53]. Further studies are needed to establish whether there is an association between these mutations and phenotypic resistance in *A. aegypti* population from Cameroon.

Conclusion

This study revealed that *A. aegypti* and *A. albopictus* are present in city of Douala with *A. aegypti* as the most abundant. Both *Aedes* species are resistance to all insecticides tested and three *kdr* mutations have been detected in *A. aegypti* samples. A full or partial recovery of susceptibility observed after pre-exposure of mosquitoes to PBO, suggests a major role of P450 genes especially *CYP6P12* and *Cyp9M6F88/87* overexpressed in resistant mosquitoes. These findings are important for the control of *Aedes* mosquitoes in the city of Douala, but more studies need to be conducted to obtain additional information on other insecticide resistance mechanisms such as metabolic resistance.

Supporting information

S1 Fig. Distribution of V1016 and 1016I alleles in *A. aegypti* population in Douala city.

QGIS version 3.14.16, was used to generate the map using open access share files (<https://gadm.org/>).

(TIF)

S2 Fig. Distribution of V410 and 410L alleles in *A. aegypti* population in Douala city.

QGIS version 3.14.16, was used to generate the map using open access share files (<https://gadm.org/>).

(TIF)

S3 Fig. Distribution of F1534 and 1534C alleles in *A. aegypti* population in Douala city.

QGIS version 3.14.16, was used to generate the map using open access share files (<https://gadm.org/>).

(TIF)

S1 Table. qPCR raw data for gene expression.

(XLSX)

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Author Contributions

Conceptualization: Aurelie P. Yougang, Charles Wondji, Basile Kamgang.

Data curation: Aurelie P. Yougang, Theodel A. Wilson-Bahun, Basile Kamgang.

Formal analysis: Aurelie P. Yougang, Christophe R. Keumeni, Theodel A. Wilson-Bahun, Basile Kamgang.

Funding acquisition: Basile Kamgang.

Investigation: Aurelie P. Yougang, Christophe R. Keumeni, Theodel A. Wilson-Bahun, Armel N. Tedjou, Basile Kamgang.

Methodology: Aurelie P. Yougang, Christophe R. Keumeni, Theodel A. Wilson-Bahun, Armel N. Tedjou, Charles Wondji, Basile Kamgang.

Project administration: Basile Kamgang.

Resources: Basile Kamgang.

Software: Aurelie P. Yougang, Christophe R. Keumeni, Theodel A. Wilson-Bahun, Basile Kamgang.

Supervision: Aurelie P. Yougang, Theodel A. Wilson-Bahun, Charles Wondji, Basile Kamgang.

Validation: Aurelie P. Yougang, Christophe R. Keumeni, Theodel A. Wilson-Bahun, Basile Kamgang.

Visualization: Aurelie P. Yougang, Theodel A. Wilson-Bahun, Charles Wondji, Basile Kamgang.

Writing – original draft: Aurelie P. Yougang, Christophe R. Keumeni, Theodel A. Wilson-Bahun, Armel N. Tedjou, Charles Wondji, Basile Kamgang.

Writing – review & editing: Aurelie P. Yougang, Christophe R. Keumeni, Theodel A. Wilson-Bahun, Armel N. Tedjou, Flobert Njiokou, Charles Wondji, Basile Kamgang.

References

1. Simo Tchegnna H, Sado Yousseu F, Kamgang B, Tedjou A, McCall PJ, et al. (2021) Concurrent circulation of dengue serotype 1, 2 and 3 among acute febrile patients in Cameroon. *PLoS Negl Trop Dis* 15: e0009860. <https://doi.org/10.1371/journal.pntd.0009860> PMID: 34695135
2. Christophers S (1960) *Aedes aegypti* (L.) the yellow fever mosquito: its life history, bionomics and structure. Cambridge University Press, London, 739 p.
3. Smith C (1956) The history of dengue in tropical Asia and its probable relationship to the mosquito *Aedes aegypti*. *J Trop Med Hyg* 59: 243–251.
4. Gratz N (2004) Critical review of the vector status of *Aedes albopictus*. *Med Vet Entomol* 18: 215–227.
5. Tedjou AN, Kamgang B, Yougang AP, Njiokou F, Wondji CS (2019) Update on the geographical distribution and prevalence of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), two major arbovirus vectors in Cameroon. *PLoS Negl Trop Dis* 13: e0007137.
6. Kamgang B, Happi JY, Boisier P, Njiokou F, Hervé JP, et al. (2010) Geographic and ecological distribution of the dengue and chikungunya virus vectors *Aedes aegypti* and *Aedes albopictus* in three major Cameroonian towns. *Med Vet Entomol* 24: 132–141.
7. Simard F, Nchoutpouen E, Toto JC, Fontenille D (2005) Geographic distribution and breeding site preference of *Aedes albopictus* and *Aedes aegypti* (Diptera: culicidae) in Cameroon, Central Africa. *J Med Entomol* 42: 726–731.
8. Kamgang B, Ngoagouni C, Manirakiza A, Nakouné E, Paupy C, et al. (2013) Temporal patterns of abundance of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) and mitochondrial DNA analysis of *Ae. albopictus* in the Central African Republic. *PLoS Negl Trop Dis* 7: e2590.
9. Kamgang B, Yougang AP, Tchoupo M, Riveron JM, Wondji C (2017) Temporal distribution and insecticide resistance profile of two major arbovirus vectors *Aedes aegypti* and *Aedes albopictus* in Yaoundé, the capital city of Cameroon. *Parasit Vectors* 10: 469.
10. Wilson-Bahun TA, Kamgang B, Lenga A, Wondji CS (2020) Larval ecology and infestation indices of two major arbovirus vectors, *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), in Brazzaville, the capital city of the Republic of the Congo. *Parasit Vectors* 13: 1–18.

11. WHO (2006) Pesticides and their application: for the control of vectors and pests of public health importance. apps. who. int/ iris/ handle/ 10665/ 69223. Accessed 18 Sept 2020.
12. Moyes CL, Vontas J, Martins AJ, Ng LC, Koo SY, et al. (2017) Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. PLoS Negl Trop Dis 11: e0005625.
13. Hemingway J, Hawkes NJ, McCarroll L, Ranson H (2004) The molecular basis of insecticide resistance in mosquitoes. Ins Biochem Mol Biol 34: 653–665. <https://doi.org/10.1016/j.ibmb.2004.03.018> PMID: 15242706
14. Ishak IH, Kamgang B, Ibrahim SS, Riveron JM, Irving H, et al. (2017) Pyrethroid resistance in Malaysian populations of dengue vector *Aedes aegypti* is mediated by CYP9 family of cytochrome P450 genes. PLoS Negl Trop Dis 11: e0005302.
15. Ishak IH, Riveron JM, Ibrahim SS, Stott R, Longbottom J, et al. (2016) The Cytochrome P450 gene CYP6P12 confers pyrethroid resistance in kdr-free Malaysian populations of the dengue vector *Aedes albopictus*. Sci Rep 6: 24707.
16. Kasai S, Komagata O, Itokawa K, Shono T, Ng LC, et al. (2014) Mechanisms of pyrethroid resistance in the dengue mosquito vector, *Aedes aegypti*: target site insensitivity, penetration, and metabolism. PLoS Negl Trop Dis 8: e2948.
17. Smith LB, Tyagi R, Kasai S, Scott JG (2018) CYP-mediated permethrin resistance in *Aedes aegypti* and evidence for trans-regulation. PLoS Negl Trop Dis 12: e0006933.
18. Badolo A, Sombié A, Pignatelli PM, Sanon A, Yaméogo F, et al. (2019) Insecticide resistance levels and mechanisms in *Aedes aegypti* populations in and around Ouagadougou, Burkina Faso. PLoS Negl Trop Dis 13: e0007439.
19. Harris AF, Rajatileka S, Ranson H (2010) Pyrethroid resistance in *Aedes aegypti* from Grand Cayman. Am J Trop Med Hyg 83: 277–284.
20. Kushwah RB, Dykes CL, Kapoor N, Adak T, Singh OP (2015) Pyrethroid-resistance and presence of two knockdown resistance (kdr) mutations, F1534C and a novel mutation T1520I, in Indian *Aedes aegypti*. PLoS Negl Trop Dis 9: e3332.
21. Kawada H, Higa Y, Futami K, Muranami Y, Kawashima E, et al. (2016) Discovery of point mutations in the voltage-gated sodium channel from African *Aedes aegypti* populations: potential phylogenetic reasons for gene introgression. PLoS Negl Trop Dis 10: e0004780.
22. Haddi K, Tomé HV, Du Y, Valbon WR, Nomura Y, et al. (2017) Detection of a new pyrethroid resistance mutation (V410L) in the sodium channel of *Aedes aegypti*: a potential challenge for mosquito control. Sci Rep 7: 46549.
23. Yougang AP, Kamgang B, Bahun TAW, Tedjou AN, Nguiffo-Nguete D, et al. (2020) First detection of F1534C knockdown resistance mutation in *Aedes aegypti* (Diptera: Culicidae) from Cameroon. Inf Dis Poverty 9: 1–12.
24. Djiappi-Tchamen B, Nana-Ndjangwo MS, Mavridis K, Talipouo A, Nchoutpouen E, et al. (2021) Analyses of Insecticide Resistance Genes in *Aedes aegypti* and *Aedes albopictus* Mosquito Populations from Cameroon. Genes 12: 828.
25. Montgomery M, Harwood JF, Yougang AP, Wilson-Bahun TA, Tedjou AN, et al. (2022) Spatial distribution of insecticide resistant populations of *Aedes aegypti* and *Ae. albopictus* and first detection of V410L mutation in *Ae. aegypti* from Cameroon. Inf Dis Poverty 11: 1–13.
26. Jupp PG (1996) Mosquitoes of Southern Africa: culicinae and toxorhynchitinae. Ekogilde Publishers.
27. WHO (2016) Entomological surveillance for *Aedes* spp. in the context of Zika virus: interim guidance for entomologists. apps. who. int/ iris/ handle/ 10665/ 204624. Accessed 21 Sept 2021.
28. Livak KJ (1984) Organization and mapping of a sequence on the *Drosophila melanogaster* X and Y chromosomes that is transcribed during spermatogenesis. Genetics 107: 611–634. <https://doi.org/10.1093/genetics/107.4.611> PMID: 6430749
29. Saavedra-Rodriguez K, Maloof FV, Campbell CL, Garcia-Rejon J, Lenhart A, et al. (2018) Parallel evolution of vgsc mutations at domains IS6, IIS6 and IIIS6 in pyrethroid resistant *Aedes aegypti* from Mexico. Sci Rep 8: 6747. <https://doi.org/10.1038/s41598-018-25222-0> PMID: 29712956
30. Saavedra-Rodriguez K, Urdaneta-Marquez L, Rajatileka S, Moulton M, Flores A, et al. (2007) A mutation in the voltage-gated sodium channel gene associated with pyrethroid resistance in Latin American *Aedes aegypti*. Ins Mol Biol 16: 785–798. <https://doi.org/10.1111/j.1365-2583.2007.00774.x> PMID: 18093007
31. Smith LB, Kasai S, Scott JG (2016) Pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus*: Important mosquito vectors of human diseases. Pestic Biochem Physiol 133: 1–12.
32. Marcombe S, Mathieu RB, Pocquet N, Riaz M-A, Poupardin R, et al. (2012) Insecticide resistance in the dengue vector *Aedes aegypti* from Martinique: distribution, mechanisms and relations with environmental factors. PLoS ONE 7: e30989.

33. Ngoagouni C, Kamgang B, Brengues C, Yahouedo G, Paupy C, et al. (2016) Susceptibility profile and metabolic mechanisms involved in *Aedes aegypti* and *Aedes albopictus* resistant to DDT and deltamethrin in the Central African Republic. *Parasit Vectors* 9: 1–13.
34. Ishak IH, Jaal Z, Ranson H, Wondji CS (2015) Contrasting patterns of insecticide resistance and knock-down resistance (*kdr*) in the dengue vectors *Aedes aegypti* and *Aedes albopictus* from Malaysia. *Parasit Vectors* 8: 1–13.
35. Ishak IH, Riveron JM, Ibrahim SS, Stott R, Longbottom J, et al. (2016) The Cytochrome P450 gene CYP6P12 confers pyrethroid resistance in *kdr*-free Malaysian populations of the dengue vector *Aedes albopictus*. *Sci Rep* 6: 1–13.
36. Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative C T method. *Nat Protoc* 3: 1101. <https://doi.org/10.1038/nprot.2008.73> PMID: 18546601
37. Chan YC, Chan KL, Ho BC (1971) *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore City. 1. Distribution and density. *Bull World Health Organ* 44: 617–627.
38. Braks MA, Honório NA, Lourenço-De-Oliveira R, Juliano SA, Lounibos LP (2003) Convergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in southeastern Brazil and Florida. *J Med Entomol* 40: 785–794.
39. Cox J, Grillet ME, Ramos OM, Amador M, Barrera R (2007) Habitat segregation of dengue vectors along an urban environmental gradient. *Am J Trop Med Hyg* 76: 820–826. PMID: 17488898
40. Hondt ON, Ntonga P, Hiol JN, Edou DN, Tonga C, et al. (2020) Adaptation compétitive d'*Aedes albopictus* Skuse, 1894 en présence d'*Aedes aegypti* Linné, 1862 dans quelques gîtes larvaires temporaires de la ville de Douala (Cameroun) dans un contexte de résistance aux pyréthrinoïdes. *Bull Soc Pathol Exot* 113: 79–87.
41. Al Nazawi AM, Aqili J, Alzahrani M, McCall PJ, Weetman D (2017) Combined target site (*kdr*) mutations play a primary role in highly pyrethroid resistant phenotypes of *Aedes aegypti* from Saudi Arabia. *Parasit Vectors* 10: 161.
42. Sombié A, Saiki E, Yaméogo F, Sakurai T, Shirozu T, et al. (2019) High frequencies of F1534C and V1016I *kdr* mutations and association with pyrethroid resistance in *Aedes aegypti* from Somgandé (Ouagadougou), Burkina Faso. *Trop Med Health* 47: 2.
43. Kamgang B, Wilson-Bahun TA, Yougang AP, Lenga A, Wondji CS (2020) Contrasting resistance patterns to type I and II pyrethroids in two major arbovirus vectors *Aedes aegypti* and *Aedes albopictus* in the Republic of the Congo, Central Africa. *Inf Dis Poverty* 9: 1–10.
44. Kasai S, Caputo B, Tsunoda T, Cuong TC, Maekawa Y, et al. (2019) First detection of a *Vssc* allele V1016G conferring a high level of insecticide resistance in *Aedes albopictus* collected from Europe (Italy) and Asia (Vietnam), 2016: a new emerging threat to controlling arboviral diseases. *Eurosurveillance* 24.
45. Ishak IH, Jaal Z, Ranson H, Wondji CS (2015) Contrasting patterns of insecticide resistance and knock-down resistance (*kdr*) in the dengue vectors *Aedes aegypti* and *Aedes albopictus* from Malaysia. *Parasit Vectors* 8: 181.
46. Mohsin M, Naz SI, Khan IA, Jabeen A, Bilal H, et al. (2016) Susceptibility status of *Aedes aegypti* and *Aedes albopictus* against insecticides at eastern Punjab, Pakistan.
47. Ngoagouni C, Kamgang B, Brengues C, Yahouedo G, Paupy C, et al. (2016) Susceptibility profile and metabolic mechanisms involved in *Aedes aegypti* and *Aedes albopictus* resistant to DDT and deltamethrin in the Central African Republic. *Parasit Vectors* 9: 599.
48. Yougang AP, Kamgang B, Tedjou AN, Wilson-Bahun TA, Njiokou F, et al. (2020) Nationwide profiling of insecticide resistance in *Aedes albopictus* (Diptera: Culicidae) in Cameroon. *Plos ONE* 15: e0234572.
49. Konan LY, Oumbouke WA, Silué UG, Coulibaly IZ, Ziogba J-CT, et al. (2021) Insecticide Resistance Patterns and Mechanisms in *Aedes aegypti* (Diptera: Culicidae) Populations Across Abidjan, Côte d'Ivoire Reveal Emergent Pyrethroid Resistance. *J Med Entomol* 58:1808–1816.
50. Kudom AA (2020) Entomological surveillance to assess potential outbreak of *Aedes*-borne arboviruses and insecticide resistance status of *Aedes aegypti* from Cape Coast, Ghana. *Acta Trop* 202: 105257.
51. Ayres CF, Seixas G, Borrego S, Marques C, Monteiro I, et al. (2020) The V410L knockdown resistance mutation occurs in island and continental populations of *Aedes aegypti* in West and Central Africa. *PLoS Negl Trop Dis* 14: e0008216.
52. Toé HK, Zongo S, Guelbeogo MW, Kamgang B, Viana M, et al. (2022) Multiple insecticide resistance and first evidence of V410L *kdr* mutation in *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) from Burkina Faso. *J Med Entomol* 36: 309–319.
53. Melo Costa M., Campos K. B., Brito L. P., Roux E., Melo Rodvalho C. et al. (2020). *Kdr* genotyping in *Aedes aegypti* from Brazil on a nation-wide scale from 2017 to 2018. *Sci Rep* 10: 1–12.