



RESEARCH ARTICLE

REVISED **Fitness cost of insecticide resistance on the life-traits of a *Anopheles coluzzii* population from the city of Yaoundé, Cameroon [version 2; peer review: 2 approved, 1 not approved]**

Diane Leslie Nkahe ^{1,2}, Edmond Kopya^{1,2}, Borel Djiappi-Tchamen^{1,3}, Wilson Toussile⁴, Nadege Sonhafouo-Chiana^{1,5}, Sevilor Kekeunou², Remy Mimpfoundi², Parfait Awono-Ambene¹, Charles Sinclair Wondji ⁶, Christophe Antonio-Nkondjio ^{1,6}

¹Malaria Research Laboratory, OCEAC, Yaoundé, Centre, PO Box 288, Cameroon

²Department of Animal Biology, University of Yaoundé 1, Yaoundé, Cameroon

³Department of Animal Biology, University of Dschang, Dschang, Cameroon

⁴Polytechnique, University of Yaoundé 1, Yaoundé, Cameroon

⁵Faculty of Sciences, University of Buea, Buea, Cameroon

⁶Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, UK

v2 First published: 20 Jul 2020, 5:171
<https://doi.org/10.12688/wellcomeopenres.16039.1>

Latest published: 08 Sep 2020, 5:171
<https://doi.org/10.12688/wellcomeopenres.16039.2>

Abstract

Background: Pyrethroid resistance is rapidly expanding in *An. gambiae* s.l. populations across Sub-Saharan Africa. Yet there is still not enough information on the fitness cost of insecticide resistance. In the present study, the fitness cost of insecticide resistance on *Anopheles coluzzii* population from the city of Yaoundé was investigated.

Methods: A resistant *An. coluzzii* colony was established from field collected mosquitoes resistant to both DDT and pyrethroid and selected for 12 generations with deltamethrin 0.05%. The Ngousso laboratory susceptible strain was used as control. A total of 100 females of each strain were blood fed and allowed for individual eggs laying, and then different life traits parameters such as fecundity, fertility, larval development time, emergence rate and longevity were measured. The TaqMan assay was used to screen for the presence of the L1014F and L1014S *kdr* mutations.

Results: Field collected mosquitoes from the F0 generation had a mortality rate of 2.05% for DDT, 34.16% for permethrin and 50.23% for deltamethrin. The mortality rate of the F12 generation was 30.48% for deltamethrin, 1.25% for permethrin and 0% for DDT. The number of eggs laid per female was lower in the resistant colony compared to the susceptible ($p < 0.0001$). Insecticide resistant larvae were found

Open Peer Review

Reviewer Status

	Invited Reviewers		
	1	2	3
version 2 (revision) 08 Sep 2020	 report		 report
version 1 20 Jul 2020	 report	 report	 report

1. **Ako Victorien Constant Edi**, Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Cote d'Ivoire
2. **Fredros O. Okumu** , Ifakara Health Institute, Ifakara, Tanzania
3. **Yaw A. Afrane** , University of Ghana,

with a significantly long larval development time (10.61 ± 0.33 days) compare to susceptible (7.57 ± 0.35 days). The number of emerging females was significantly high in the susceptible group compared to the resistant. The adults lifespan was also significantly high for susceptible (21.73 ± 1.19 days) compared to resistant (14.63 ± 0.68 days). Only the L1014F-*kdr* allele was detected in resistant population..

Conclusion: The study suggests that pyrethroid resistance is likely associated with a high fitness cost on *An.coluzzii* populations. The addition of new tools targeting specifically larval stages could improve malaria vectors control and insecticide resistance management.

Keywords

life-traits, *An. coluzzii*, insecticide resistance, fitness cost, Yaoundé, Cameroon

Accra, Ghana

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Christophe Antonio-Nkondjio (antonio_nk@yahoo.fr)

Author roles: **Nkahe DL:** Data Curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – Original Draft Preparation; **Kopya E:** Data Curation, Investigation, Methodology; **Djiappi-Tchamen B:** Investigation; **Toussile W:** Formal Analysis, Methodology; **Sonhafouo-Chiana N:** Investigation; **Kekeunou S:** Writing – Review & Editing; **Mimpfoundi R:** Writing – Review & Editing; **Awono-Ambene P:** Writing – Review & Editing; **Wondji CS:** Supervision, Writing – Review & Editing; **Antonio-Nkondjio C:** Conceptualization, Funding Acquisition, Project Administration, Supervision, Validation, Visualization, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was funded by Wellcome [202687; Senior Fellowship in Public Health and Tropical Medicine to CAN]. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

Copyright: © 2020 Nkahe DL *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Nkahe DL, Kopya E, Djiappi-Tchamen B *et al.* **Fitness cost of insecticide resistance on the life-traits of a *Anopheles coluzzii* population from the city of Yaoundé, Cameroon [version 2; peer review: 2 approved, 1 not approved]** Wellcome Open Research 2020, 5:171 <https://doi.org/10.12688/wellcomeopenres.16039.2>

First published: 20 Jul 2020, 5:171 <https://doi.org/10.12688/wellcomeopenres.16039.1>

REVISED Amendments from Version 1

In the present version of the manuscript we took into consideration remarks made by the reviewers to improve on the quality of the paper. Correction on calculations and a paragraph in the discussion was added.

Any further responses from the reviewers can be found at the end of the article

Introduction

Malaria prevention mainly relies on the use of vector control measures with indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) as the core interventions¹. Five insecticide families, organophosphates, organochlorines, carbamates, pyrethroids and neonicotinoid are used in public health². The massive use of insecticides particularly pyrethroids over the last decades in vector control and in agriculture resulted in rapid expansion of insecticide resistance, which now affects almost all insecticides³. Several mechanisms, including metabolic detoxification, target site mutations and cuticular genes are responsible for insecticide resistance^{4,5}. Most common mechanisms associated with insecticide resistance in *Anopheles gambiae* s.l. include target-site resistance, notably knockdown resistance (kdr mutation with the I014F and I014S alleles responsible for resistance to DDT and pyrethroids) and the acetylcholinesterase (Ace-1) G119S mutation responsible for resistance to organophosphates and carbamates⁵⁻⁷. Metabolic resistance is another major resistance mechanism, occurring through the upregulation of several detoxification genes from three main families, the esterases, cytochrome P450 monooxygenases, and glutathione S-transferases responsible for resistance to different insecticide families and pollutants⁴. There is a growing concern about the negative impact that insecticide resistance could have on malaria control. It increases the survival rate of mosquitoes exposed to insecticides in treated areas which could potentially lead to greater population size, increase in mosquito burden and diseases transmission^{8,9}. Studies conducted so far suggested that resistant alleles could be associated with negative pleiotropic effects that could affect mosquito fitness, vectorial competency and disrupts the normal physiological functions of the mosquito^{10,11}. Insecticide resistance has always been associated with lower fecundity, longer developmental time, reduced longevity, and lower mating success^{12,13}. With the increasing use of insecticides, mosquitoes have been reported over recent years to have become multiresistant to different insecticide compounds¹⁴⁻¹⁷. Understanding the influence of insecticide resistance on vector population dynamic is becoming crucial for the implementation of insecticide resistance management strategies. Although resistance is largely expanding in *An. gambiae* s.l. populations from Cameroon, there has been so far little information on the influence of pyrethroid resistance on *An. gambiae* s.l. fitness. Experimental infection studies comparing resistant *versus* susceptible colonies have suggested increased prevalence of *Plasmodium* infections in *An. gambiae* s.s. resistant strains¹⁸⁻²⁰. Studies on the malaria vector *An. funestus* s.s. indicated that the presence of the L119F-GSTe2 resistant allele was associated with reduced fecundity, increased

larval developmental time and adult longevity²¹. Further analysis suggested that this mechanism could also influence the vectorial capacity of resistant *An. funestus* s.s. populations²². During the last decade important changes have been reported in *An. gambiae* s.l. populations from the city of Yaoundé with populations becoming increasingly tolerant to organic pollution²³, more resistant to pyrethroids and to different compounds^{14,24,25}, changes in the biting behavior was also reported²⁶. Yet the influence of these changes on the vectorial capacity or the fitness of *An. gambiae* s.l. populations has not been fully addressed.

In the present study an insecticide resistant *An. coluzzii* colony from the city of Yaoundé, was compared to a susceptible *An. coluzzii* laboratory colony “the Nguosso colony” to determine life-traits parameters affected by the increased expansion of insecticide resistance in this vector population.

Methods

Study site

The study was conducted in the city of Yaoundé, the capital of Cameroon (3° 52' 12 N; 11° 31' 12 E) from September 2018 to September 2019. In Yaoundé, *An. gambiae* s.l. is the main malaria vector. In order to obtain representative sample of the resistant *An. gambiae* s.l. population from the city of Yaoundé, anopheline larvae used to build the colony, were collected from different districts and locations (Tsinga, Nsam, Nkolbisson, Obobogo, Mvog-beti, Nouvelle Route Bastos, Nouvelle Route Tam-Tam, and Nouvelle Route Nkoldongo). In Yaoundé, malaria is highly prevalent, with the transmission rate varying from 0 to 90 infected bites/man/year²⁵. LLINs is the main method used by the population to prevent from malaria transmission²⁷. According to recent record it is estimated that over 75% of households in Yaoundé own at least a net²⁸. Urban agriculture is also practiced on a large scale in the city and large quantities of pesticides are used by farmers and these, alongside the use of LLINs, are the main sources of insecticide selection for mosquitoes in the city^{29,30}.

Susceptible strain

The laboratory colony used in the study is the Nguosso strain, originating from the district of Nguosso in Yaoundé, reared since 2006. This Nguosso *An. coluzzii* strain is fully susceptible to both permethrin and deltamethrin (mortality rate: ≥98% after one hour exposition to WHO impregnated papers).

Susceptibility assays and establishment of a resistant laboratory colony

Anopheline larvae were collected in standing water collections on the field. Once in the laboratory, larvae were pooled and reared at mean temperature of 30°C and 73–75% relative humidity. After emergence, males were separated from females and adult females aged 3 to 5 days were used to conduct bioassays with deltamethrin (0.05%), permethrin (0.75%), DDT (4%), bendiocarb (0.1%) and Malathion (5%) according to WHO guidelines¹³.

The resistant *An. coluzzii* colony was established by regular selection (once every two generations) of mosquitoes, exposing 3 to 5 day-old unfed females and males to 0.05% deltamethrin

for 12 generations. Batches of 20 to 25 mosquitoes per tube were exposed to 0.05% deltamethrin-impregnated papers for 1 hour. Bioassays were conducted at temperatures of $25\pm 2^{\circ}\text{C}$ and 70–80% relative humidity. Control tests were conducted using untreated papers. 24 hours after exposure, survivor male and female were pooled for the mating and fed with a 5% glucose solution. After selection, the susceptibility status of the F12 generation was checked for the following insecticides: permethrin (0.75%), DDT (4%), bendiocarb (0.1%), malathion (5%) and PBO (4%) in order to check the implication of P450 metabolic-based mechanisms.

Isofemale rearing and life-trait assessment

Blood meal

To ensure that mosquitoes would feed, the glucose solution was removed 24 hours before blood feeding. Anopheline aged 3 to 6 days were placed into three cages (30×30×30 cm) of 100 females each for each strain and blood fed for 20 minutes on an anaesthetized rabbit. After blood feeding, females were provided with glucose solution, to allow maturation of eggs. The engorgement rate was assessed by counting well fed females.

The study was conducted under the ethical clearance N° 2016/11/832/CE/CNERSH/SP delivered by the Cameroon National Ethics (CNE) Committee for Research on Human Health Ref D30-172/L/MINSANTE/SG/DRS/TMC of 04 April 2017.

Fecundity and fertility

For each strain (resistant and susceptible), 100 gravid females were placed in individual cups with damp filter paper to enable them lay eggs. After oviposition, the number of eggs laid per female was counted under a stereo microscope and eggs batch from each female were placed in water (plastic basins (17×12×6.50 cm) containing 200 ml of fresh water) 24 hours after the day of oviposition. All females that laid eggs were counted and stored at -20°C into numbered eppendorf tubes containing desiccant for further analyses. The egg from each isofemale line was reared separately.

Larval development

To reduce competition, a maximum of 50 larvae were reared per tray. Additional trays were used for females with more than 50 larvae. Larvae were fed using baby fish food (TetraMin) under standard insectary conditions. During the rearing process, water from the tray was replaced every two days to reduce the influence of evaporation or pollution. At the pupal stage, the number of pupae was recorded every day and they were transferred into paper cup (8.50×10×7cm), up to 30 pupae were placed per cup. Different information were recorded from each colony reared, the number of individual per larval stage, the length of larval development, the number of larvae reaching the pupae stage, the number of pupae emerging as adults and the sex ratio.

Adult longevity

After emergence, the number of males and females was recorded in each cup. Adult mosquitoes were fed with a 5% glucose solution. Each female progeny was placed in separate cup

and followed. The survival rate was assessed by recording the number of males and females dying each day in each cup. Dead mosquitoes were removed from each cup daily for each family and kept at -20°C in 1.5-ml Eppendorf tubes containing desiccant.

Molecular identification

Genomic DNA was extracted from whole mosquitoes using a previously described protocol³¹. Molecular identification analyses were performed following the SINE200 PCR method described by Santolamazza *et al.*³² to identify species of the *An. gambiae* complex.

Detection of knockdown resistance (*kdr*)

The presence of the *kdr* allele was checked in field collected mosquitoes (F0) and the laboratory resistant colony (F12 generation). A set of 119 females of the F0 generation and 111 F12 generation were randomly selected in each group for *kdr* analysis. L1014F and L1014S mutations were screened using the Mx3005P Real-Time PCR System, as describe previously³³. The primers *kdr*-Forward (5'-CAT TTT TCT TGG CCA CTG TAG TGA T-3') and *kdr*-Reverse (5'-CGA TCT TGG TCC ATG TTA ATT TGC A-3') were used. The probes *kdr*W (5'-ACG ACA AAA TTT C-3') and *kdr*E (5'-ACG ACT GAA TTT C-3') labelled with fluorochrome FAM, were used to detect the mutant alleles (1014F and 1014S) while the probe Wildtype (5'-CTT ACG ACT AAA TTT C-3') labelled with fluorochrome HEX, were used for the wild type susceptible alleles detection. The Master Mix solution (9 μl) contained 5 μl of Sensimix (Biogen), 0.13 μl of each probe coupled to allelic specific primer and 3.88 μl of water. The thermocycler was set to run samples following the temperature cycling condition of: 1 hold of 10 minutes at 95°C for initial denaturation, followed by 40 cycles each of 95°C for 10 seconds, and 60°C for 45 seconds. For each experiment, there was one homozygote resistant for L1014S and L1014F *kdr*; one heterozygote for L1014S and L1014F *kdr*; and one susceptible L1014L used as positive controls. The negative controls were wells with 1 μl of ddH₂O.

Statistical analysis

Statistical analyses were performed with the software Excel 2010 and the statistical analysis software R (version 4.0.0) via RCommander (Rcmdr Version 2.6-2) and RStudio (version 1.2.5042). The Shapiro-Wilk test was applied to assess whether eggs and larvae counts conform to a normal distribution. Comparison of proportions between the two strains was conducted using a chi-square (χ^2) test. Female fecundity and fertility was assessed by comparing respectively the oviposition and the hatching rate between both strains using Welch's two-sample t-test. The life-trait parameters such as duration of larval development and longevity were assessed by comparing means of both susceptible and resistant strains using a Kruskal-Wallis non-parametric test. While those such as pupation, emergence, sex-ratio were assessed by comparing means of both strains using Welch's two sample t-test. To draw survival curves or Kaplan-Meier curve of larvae and adults, we used the package 'survminer' version 0.4.6 that contains the function 'ggsurvplot ()'. The latest allow drawing curve with the 'number at risk' table and the 'censoring count plot'. The level of significance of each test was set at $\alpha < 0.05$. Scatter

plots were obtained from genotypes scored using the MxPro-MX3005P qPCR Software and the fluorescence (ΔR) threshold adjusted manually for each dye, if necessary, to enable the correct scoring of positive controls. The allele frequency of individuals carrying the *kdr* mutation was calculated using the formula $f(R) = (2 \times RR + RS)/2N$, with RR = total number of homozygote resistant; RS = total number heterozygote resistant; N = total number of mosquitoes screened for the *kdr* mutation. Genotype frequency was calculated as relative frequencies of the homozygote resistant and heterozygote resistant individuals.

Results

Blood feeding rate

In the susceptible colony, 93% of females successfully blood fed (280/300), while only 34% females successfully blood fed in the resistant colony (102/300), revealing a significant difference in blood feeding between the two colonies ($\chi^2 = 147.68$, $df = 1$, $P < 0.0001$) (Table 1).

Fecundity and fertility

After choosing 100 fully blood fed females of each strain for individual egg laying, in the susceptible group, 86 females laid. A total of 9604 eggs, corresponding to a mean of 111.67 ± 5.36 eggs/female was recorded (Table 2). In the resistant colony, 40 females laid 3736 eggs (Table 1), which correspond to an average of 93.33 ± 10.77 eggs/female for females which were able to lay (Table 2). In the resistant and the susceptible colonies, eggs count was found in conformity with a normal distribution Shapiro-Wilk normality test (resistant: $W = 0.95$, $P = 0.06$, susceptible: $W = 0.97$, $P = 0.05$).

Comparison between the two groups resistant vs susceptible indicated that the average number of eggs laid by resistant females was statistically lower (Kruskal-Wallis $\chi^2 = 42.86$, $df = 1$, $P = 5.89 \times 10^{-11}$). Fecundity parameters recorded were significantly higher in the susceptible colony compare to the resistant colony ($\chi^2 = 43.44$, $df = 1$, $P = 2.19 \times 10^{-11}$) (Table 2). The mean number of larvae was statistically lower

Table 1. Comparison of the blood feeding rate, fecundity and fertility rate of susceptible vs resistant mosquitoes.

Parameters	Susceptible strain		Resistant strain		P-value
	n/N	Rates (%)	n/N	Rates (%)	
Blood feeding	280/300	93.33*	102/300	34*	$p < 0.0001$
Laying	86/100	86*	40/100	40*	$p < 0.0001$
Hatching rate	7101/9604 (larvae/eggs)	73.94*	2459/3736 (larvae/eggs)	65.82*	$p < 0.0001$

Abbreviations: N: total number of mosquitoes initially allowed to blood feed and lay eggs; n: number of mosquitoes who successfully blood fed and laid eggs; * significantly different ($P < 0.05$).

Table 2. Differences in life-traits parameters between susceptible and resistant *Anopheles coluzzii*.

Parameters	Susceptible strain		Resistant strain		chi-squared & t test	P-value
	N	Means \pm SE	N	Means \pm SE		
Eggs	9604	$111.67 \pm 5.36^*$	3736	$93.33 \pm 10.77^*$	42.86	$p < 0.0001$
Larvae	7101	$83.54 \pm 4.76^*$	2459	$63.05 \pm 9.49^*$	2.78	0.004
Larval development time (days)	-	$7.57 \pm 0.18^*$	-	$10.61 \pm 0.17^*$	-	0.035
Pupae	5434	63.19 ± 4.37	1976	49.40 ± 7.7	-1.18	0.12
Emergence	5226	54.26 ± 6.76	1907	47.68 ± 7.48	-0.33	0.74
Dead	238	2.7 ± 0.39	61	1.53 ± 0.29	1.26	0.21
Males	2530	26.43 ± 3.31	982	24.55 ± 3.78	-2.89	0.15
Females	2696	$28.10 \pm 3.53^*$	925	$23.13 \pm 3.75^*$	3.10	0.032
Adult live span	Male	$20.77 \pm 0.52^*$	-	$15.35 \pm 0.41^*$	1696	$p < 0.0001$
	Female	$21.71 \pm 0.47^*$	-	$13.90 \pm 0.46^*$	4350	$p < 0.0001$

Means \pm standard error is shown in all cases. Numbers followed by the star differ significantly ($P < 0.05$); N: number.

in the resistant colony compare to the susceptible (Table 2) (Welch Two Sample t-test: $t = 2.78$, $df = 53$, $P = 0.004$).

Larval and pupae development

The average length of larval development from hatching to pupation was compared between the two colonies. The average length of larval development to the pupa stage was 7.57 days for the susceptible and 10.61 days for the resistant group (Figure 1). Survival probability analysis indicated that, resistant larvae take much more time to arrive to the pupa stage compared to the susceptible ($\chi^2 = 2251$, $df = 1$, $P = <2 \times 10^{-16}$). In the susceptible group, high pupation rate was recorded between days 7 and 8, whereas for resistant larvae a peak of pupation was detected on day 10 (Figure 1).

Mean number of pupae and emergence rate in susceptible and resistant strain

In the susceptible colony, out of 7101 first instars larvae, 5434 larvae successfully arrived at the pupae stage and 5226 emerged as adults. The following correspond to 76.52% getting to the pupae stage and 73.59% to the adult stage. Within resistant out of 2459 larvae of the first instar, 1976 successfully arrived at the pupa stage (80.36%) and 1907 (77.55%) emerged as adults. The average number of offspring pupae per female was not significantly different between the two groups (Welch two-sample t-test: $t = -1.18$, $df = 95.16$, $P = 0.12$) (Table 2). The average number of adults emerging was also not significantly different between the two groups (Welch two-sample t-test: $t = -0.67$, $df = 82.33$, $P = 0.25$) (Table 2). Mortality during emergence was similar between susceptible and resistant groups (Welch two-sample t-test: $t = 1.26$, $df = 54.34$, $P = 0.10$) (Table 2).

Sex-ratio of adults mosquitoes

Of the 5226 mosquitoes who successfully emerged as adults in the susceptible group, 48.41% ($N=2530$) were males and 51.59% ($N=2696$) were females. Of the 1907 mosquitoes who successfully emerged as adults in the resistant group, there was 51.49% ($N=982$) of males and 48.51% ($N=925$) females. The proportion of emerging female was statistically similar to that

of male in each offspring group, susceptible colony ($\chi^2 = 0.17$, $df = 1$, $P = 0.68$) and resistant colony ($\chi^2 = 0.06$, $df = 1$, $P = 0.81$). There were significantly more susceptible female than resistant female in the offspring (Welch two-sample t-test: $t = 3.10$, $df = 61.99$, $P = 0.001$) (Table 2).

Life span of the progeny of susceptible and resistant adults mosquitoes

The life span after emergence of the progeny of resistant and susceptible mosquitoes was assessed. Susceptible individuals appeared to live longer than resistant individuals (Figure 2A) ($\chi^2 = 4350$, $df = 1$, $P = <2 \times 10^{-16}$). The average life span for susceptible individuals was similar between males (20.76 ± 0.52 days) and females (21.71 ± 0.47 days), ($t = -1.33$, $df = 81$, $p = 0.18$). In the resistant group, females had a significantly shorter life span (13.90 ± 0.46 days) than males (15.35 ± 0.41 days), ($t = 2.35$, $df = 30$, $p = 0.02$), (Table 2). The difference between the average life span number of males and females in each group was not significant for the susceptible group ($t=2.58$, $df = 81$, $p = 0.9$) whereas it was significant for the resistant group ($t = 2.75$, $df = 30$, $p = 0.005$). (Figure 2B).

Insecticide resistance profile of F0 and F12 generation

Field-collected mosquitoes from the F0 generation had a mortality rate of 2.05% [0.55-3.56] for DDT, 32.16% [29.94-38.37] for permethrin, 50.23% [46.37-54.10] for deltamethrin, 96.42 [96.38-96.48] for bendiocarb and 100% for malathion. The high resistance status of the strain to deltamethrin was maintained through generations, with the mortality rate decreasing from $50.23 \pm 3.86\%$ for the F0 generation to $30.48 \pm 6.23\%$ for the F12 generation. In the same way, the F12 generation showed 0% mortality to DDT 4%, 1.25% mortality rate to permethrin 0.75%, no change in their susceptibility to bendiocarb 0.1% (mortality rate: $95 \pm 1.35\%$) and malathion 5% (mortality rate: 100%) (Figure 3A). When mosquitoes of the F12 generation were preexposed to PBO a mortality rate of $67.50 \pm 6.87\%$ to deltamethrin 0.05% was recorded whereas, no variation in the mortality to permethrin 0.75% was recorded. (Figure 3B).

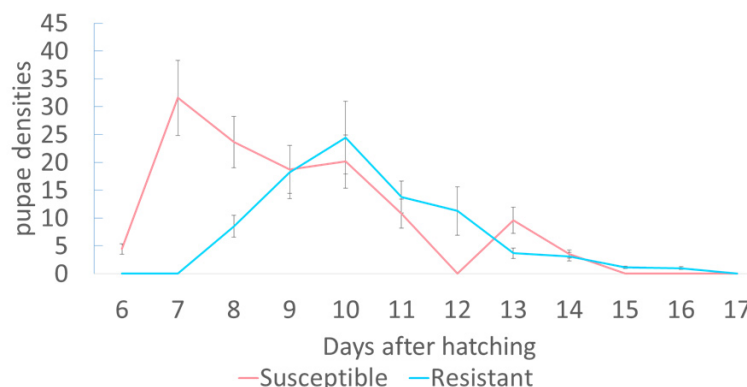


Figure 1. Mean of larval development time of susceptible and resistant larvae. Error bars = standard deviation.

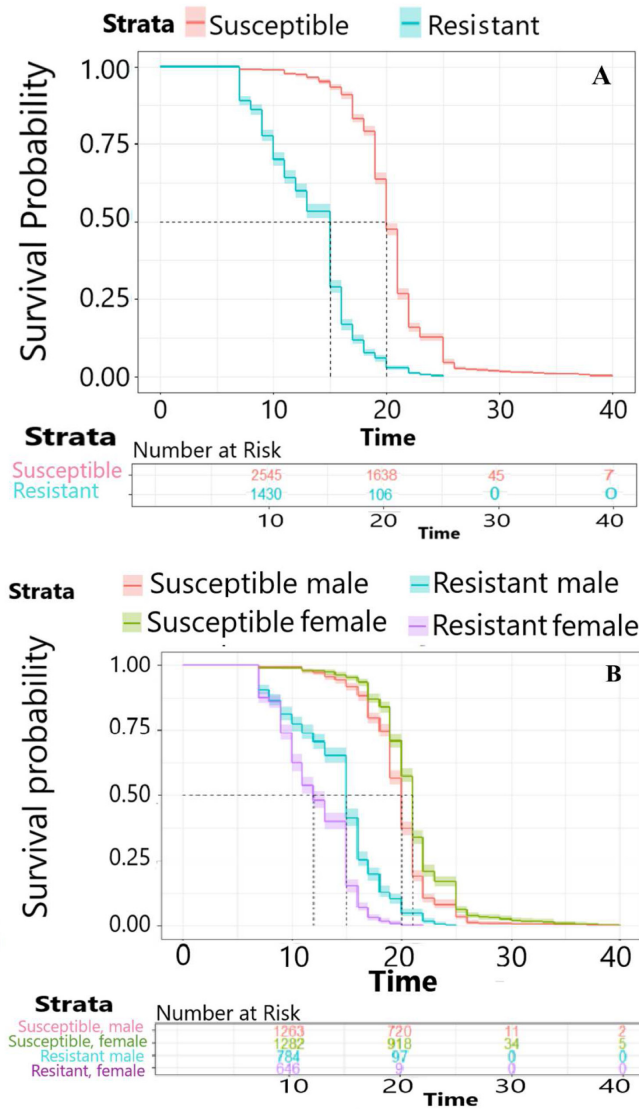


Figure 2. Life span and survival probabilities of mosquitoes. Comparison of the adult life span of susceptible and resistant mosquitoes (A); Survival probability of male and female of susceptible and resistant mosquitoes (B). X-axis = time in days, Y-axis = probability of surviving. Lines = survival curves of different groups (strata). Vertical tick mark = half live time of each group.

Molecular identification and *kdr* detection

Molecular identification performed on field-collected mosquitoes (F0 generation) showed that 7.53% (7/93) belong to *An. gambiae* and 92.47% (86/93) were *An. coluzzii*. While identification of 103 susceptibles and 311 resistants (F12 generation) mosquitoes confirms that the two groups belong to *An. coluzzii* species. The *kdr* allele 1014F was detected both in the field collected and the resistant group. The field-collected mosquitoes (F0) revealed 24/119 were homozygotes resistant, 90/119 were heterozygotes and 5/119 were homozygotes susceptibles, while in the F12 generation, all 111 individuals had the *kdr* West allele 1014F to make them homozygote resistant. The frequency of the L1014F *kdr* mutation was 58% for the F0 generation and 100% for the F12 generation. The 1014S allele was not detected (Table 3).

Raw data for this study are available, see *Underlying data*³⁴.

Discussion

Insecticide resistance is rapidly expanding in *An. gambiae* s.l. population from the city of Yaoundé^{14,35}. Yet the influence of insecticide resistance on *An. gambiae* s.l. species life trait and evolution is not well understood³⁶. The present study was undertaken to assess the influence of insecticide resistance on the fitness and life trait of *An. coluzzii* population by comparing a susceptible to a resistant colony. The study indicated high fitness cost in the resistant compared to the susceptible colony. This was consistent with previous observations done on the vector *An. funestus* s.s. in Cameroon^{11,20}. Mosquitoes in the city of Yaoundé have been reported to be resistant to pyrethroids, and also to a large set of compounds including

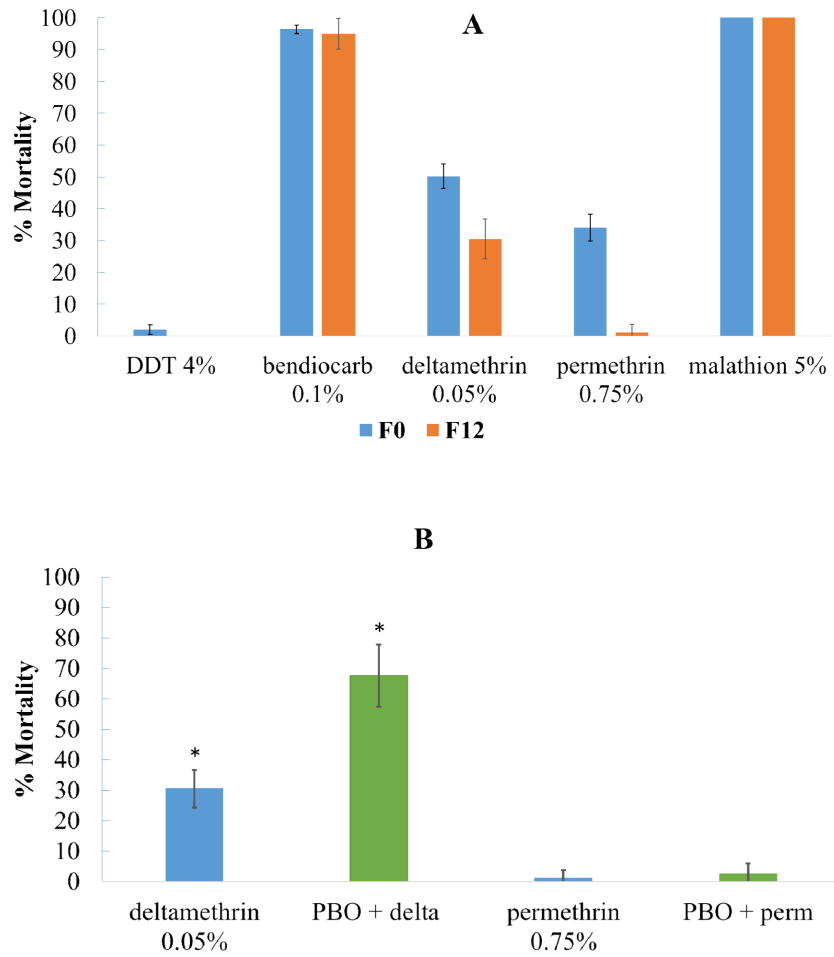


Figure 3. Mortality rates following insecticide exposure. Mortality of F0 and F12 generation to different insecticides (A). Susceptibility status of F12 generation after preexposure to PBO (B). * P<0.05, PBO+delta=PBO 4% + deltamethrin 0.05%, PBO+perm=PBO 4% + permethrin 0.75%. Error bars= 95% confidence intervals.

Table 3. Genotypes and alleles frequencies of 1014F *kdr* mutation in resistant *An. coluzzii* populations.

Generation	Genotypes						Alleles	
	RR		RS		SS		2N	f(R)
	n/N	%[95%CI]	n/N	%[95%CI]	n/N	%[95%CI]		
F0	24/119	21.17 [14.29-29.11]	90/119	75.63 [67.92-83.34]	5/119	4.20 [0.56-7.80]	238	0.58
F12	111/111	100	0	0	0	0	222	1
P-value		P < 0.0001						

Abbreviations: RR: homozygous resistant; RS: heterozygous 1014F; SS: homozygous susceptible f(): frequency of the allele; [95%CI]: 95% confidence interval; N: total number of mosquitoes initially processed; n: number of mosquitoes successfully screened for the *kdr* mutation; F0: field collected population; F12: population selected to deltamethrin 0.05% for 12 generations.

DDT, carbamate and pollutants^{24,30,35,37}. Comparison of many life-trait parameters between the two colonies indicated differences at different levels. The blood feeding success was high in susceptible compare to resistant. The difference could have resulted from the fact that the susceptible colony was more adapted to blood feeding on rabbit blood as it has been maintained in laboratory for almost 14 years, whereas this is not the case for the resistant colony which has just been colonized in the insectary. Similar observations have been reported elsewhere¹². According to Martins *et al.*, the blood meal is a key parameter which directly affects the general fitness, since it influences the number of eggs laid¹². Eggs from susceptible *An. coluzzii* also showed a high hatching rate and viability compared to those from resistant *An. coluzzii*. The results somewhere suggest that the rate of insemination could be lower in the resistant strain compared to the susceptible. Fecundity and fertility were also found to be reduced in resistant *An. funestus*²¹, it is likely that this could be associated with resistance phenotype. Briegel *et al.* demonstrated that fecundity increases with successive blood meal, for *An. gambiae* s.l. it increases to 50% when two blood meals are provided for a single gonotrophic cycle³⁸.

The length of larval development in the resistant colony was three days longer than that of susceptible, similar findings have been reported for *An. funestus* s.s.²¹. The longer the larval development time suppose higher exposition to pollutants and physico-chemical parameters from the breeding sites and more vulnerability to natural predators. A shorter development time is likely to accelerate adult emergence, leading to increase vector density, which is an important parameter of the vectorial capacity. In previous laboratories studies, Grimmig *et al.* suggested that extended larval development time for *An. gambiae* s.l. could result from high larval densities³⁹, but such a hypothesis is excluded here because the density of larvae per tray was below 50, which is sufficient for a good growth. The longer development time likely translates the influence of insecticide resistance on the general metabolism of resistant mosquitoes. Increased deleterious effects on the development, associated to pyrethroid resistance, have been demonstrated in *Aedes aegypti*^{12,40}. Tchouakui *et al.* also reported a longer developmental time for *An. funestus* s.s. larvae carrying the 119F-GSTe2 and the CYP6P9a-R resistant allele compared to those with susceptible allele in Cameroon^{11,21}. The expression of resistance genes has also been reported to induce alteration of some functions such as larval motility which could alter their capacity to look for food^{41,42}.

The density of pupae and the transition to adult stage were not significantly different between the two strains, suggesting that fitness cost mostly affects the larval stage and has no visible effect on pupae and adult emergence. The following could derive from the fact that pupae do not feed and there is limited influence of exogenic factors such as density or nutrient on this stage^{39,43,44}. No significant distortion of the sex ratio was recorded neither in the susceptible nor in the resistant colony. The absence of difference at this level,

particularly for the resistant colony, could derive from the reduce number of larvae succeeding at the pupae and adult stage. In the nature, one male can inseminate several females during its lifespan, while females just need a unique insemination to accomplish multiple gonotrophic cycle for the rest of their life⁴⁵. Therefore, the number of females reaching the adult stage is an important parameter of reproduction success and vectorial capacity whereas this is not true for males⁴⁵.

Despite a similar life span between males and females in each colony, the progeny of susceptible mosquitoes was found to live longer in general 6 to 7 days longer than resistant mosquitoes. To be able to transmit malaria parasites it is important that the mosquitoes live long enough to enable the extrinsic development of the parasite in the mosquito which last approximately 12 days. From the study, it clearly appeared that if resistant mosquitoes are not infected after their first blood meal, few are going to be involved in malaria transmission since the average life span of resistant mosquitoes is estimated to be about 14 days for females. Vectors longevity is considered as a key factor contributing to the vectorial capacity of mosquitoes in endemic settings^{18,21}. It is possible that the reduced life span of resistant *An. gambiae* s.l. mosquitoes in Yaoundé is negatively influencing its vectorial capacity. Reduced vectorial capacity associated with shorter longevity has previously been reported for pyrethroid-resistant *Aedes aegypti* populations in Brazil and in Thailand^{12,40,46}. As oppose to these findings, increased longevity, an increased vectorial competency was reported for F1 resistant *An. funestus* s.s. possessing the 119F-GSTe2 allele^{21,47}. It is possible that in the present situation, the resistance status of *An. coluzzii* to insecticides also affects its vectorial competence. These findings still need to be validated by extensive field studies and experiments. Although the use of pyrethroid treated nets are considered to induce a low mortality in resistant individuals, resistant mosquitoes were however found to exhibit reduce blood feeding rate, low fecundity and short adult survival rate all this somewhere suggest a long term impact of insecticide base intervention (such as pyrethroid treated nets) selection on vector population. This long term negative impact confirm continuous performance of pyrethroid treated nets interventions on vector populations. This unrecognized impact of treated nets need to be highlighted in different epidemiological settings. Out of the two species *An. gambiae* and *An. coluzzii* identified in F0 generation, only *An. coluzzii* has successfully been maintained in laboratory across generations. The disappearance of the *An. gambiae* species could be explained by the low number of specimens and probably to the low adaptation capacity of the species to laboratory conditions. Molecular analysis of the resistant colony suggested the exclusive presence of the *kdr* West allele (1014F) at a high rate in the resistant colony. As mentioned in previous studies, the *kdr* mutation alongside metabolic detoxification could be the main mechanisms involved in pyrethroid resistance in *An. coluzzii* from Yaoundé^{16,48,49}. Detoxification genes such as *Cyp6p3*, *Cyp6m2* and *Cyp9k1*, have been reported involved in pyrethroid

resistance in *An. gambiae* s.s. and *An. coluzzii* populations from Yaoundé^{30,35,50}.

Conclusion

The study suggests that increase expansion of insecticide resistance in *An. coluzzii* populations from the city of Yaoundé, is likely associated with accumulation of deleterious effects affecting the life-traits of *An. coluzzii*. It appears from the study that the longer development time could render resistant larvae more vulnerable to control measures such as larviciding and to predators. It also appeared that adult resistant mosquitoes are associated with reduce fecundity, blood feeding rate and short survival rate, all these could affect adult vectorial capacity. Data generated from the present study, could be used to improve vector control strategies to be implemented on the field.

Data availability

Underlying data

Open Science Framework: Nkahe *et al.* 2020. <https://doi.org/10.17605/OSF.IO/C8EUX>³⁴.

This project contains the following underlying data:

- Data on life-traits (XLSX). (Trait data from the mosquitoes captured during this study.)
- pcr leslie (XLSX). (PCR data from this study.)
- Data Dictionary (DOCX).

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

References

- World Health Organization: **World malaria report 2018**. World Health Organization: Geneva. 2018. [Reference Source](#)
- World Health Organization: **List of WHO prequalified vector control products**. World Health Organization: Geneva. 2017.
- Ranson H, N'guessan R, Lines J, *et al.*: **Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control?** *Trends Parasitol.* 2011; **27**(2): 91–98. [PubMed Abstract](#) | [Publisher Full Text](#)
- Hemingway J: **The molecular basis of two contrasting metabolic mechanisms of insecticide resistance**. *Insect Biochem Mol Biol.* 2000; **30**(11): 1009–1015. [PubMed Abstract](#) | [Publisher Full Text](#)
- WHO: **Global plan for insecticide resistance management in malaria vectors (GPIRM)**. World Health Organization Geneva. 2012. [Reference Source](#)
- Elanga-Ndille E, Nouage L, Ndo C, *et al.*: **The G119S Acetylcholinesterase (Ace-1) Target Site Mutation Confers Carbamate Resistance in the Major Malaria Vector *Anopheles gambiae* from Cameroon: A Challenge for the Coming IRS Implementation**. *Genes (Basel).* 2019; **10**(10): 790. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Vontas J, Grigoraki L, Morgan J, *et al.*: **Rapid selection of a pyrethroid metabolic enzyme CYP9K1 by operational malaria control activities**. *Proc Natl Acad Sci U S A.* 2018; **115**(18): 4619–24. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Alout H, Roche B, Dabiré RK, *et al.*: **Consequences of insecticide resistance on malaria transmission**. *PLoS Pathog.* 2017; **13**(9): e1006499. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Wondji CS, Coleman M, Kleinschmidt I, *et al.*: **Impact of pyrethroid resistance on operational malaria control in Malawi**. *Proc Natl Acad Sci U S A.* 2012; **109**(47): 19063–19070. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Raymond M, Berticat C, Weill M, *et al.*: **Insecticide resistance in the mosquito *Culex pipiens*: what have we learned about adaptation?** *Microevolution Rate, Pattern, Process.* 2001; **8**: 287–96. [Publisher Full Text](#)
- Tchouakui M, Riveron JM, Miranda J, Mugenzi LMJ, *et al.*: **Cytochrome P450 metabolic resistance (CYP6P9a) to pyrethroids imposes a fitness cost in the major African malaria vector *Anopheles funestus***. *Heredity (Edinb).* 2020; **124**(5): 621–32. [PubMed Abstract](#) | [Publisher Full Text](#)
- Martins AJ, Dutra e Mello Ribeiro C, Bellinato DF, *et al.*: **Effect of insecticide resistance on development, longevity and reproduction of field or laboratory selected *Aedes aegypti* populations**. *PLoS One.* 2012; **7**(3): e31889. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- World Health Organization: **Test procedures for insecticide resistance monitoring in malaria vector mosquitoes 2nd ed.** World Health Organization, Geneva, Switzerland. 2016. [Reference Source](#)
- Antonio-Nkondjio C, Sonhafouo-Chiana N, Ngadjou CS, *et al.*: **Review of the evolution of insecticide resistance in main malaria vectors in Cameroon from 1990 to 2017**. *Parasit Vectors.* 2017; **10**(1): 472. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Awolola TS, Oduola OA, Strode C, *et al.*: **Evidence of multiple pyrethroid resistance mechanisms in the malaria vector *Anopheles gambiae* sensu stricto from Nigeria**. *Trans R Soc Trop Med Hyg.* 2009; **103**(11): 1139–1145. [PubMed Abstract](#) | [Publisher Full Text](#)
- Fadel AN, Ibrahim SS, Tchouakui M, *et al.*: **A combination of metabolic resistance and high frequency of the 1014F *kdr* mutation is driving pyrethroid resistance in *Anopheles coluzzii* population from Guinea savanna of Cameroon**. *Parasit Vectors.* 2019; **12**(1): 263. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Djouaka R, Riveron JM, Yessoufou A, *et al.*: **Multiple insecticide resistance in the major malaria vector *Anopheles funestus* in southern Ghana: implications for malaria control**. *Parasites Vectors.* 2016; **9**: 453. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Alout H, Ndam NT, Sandeu MM, *et al.*: **Insecticide resistance alleles affect vector competence of *Anopheles gambiae* s.s. for *Plasmodium falciparum* field isolates**. *PLoS One.* 2013; **8**(5): e63849. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kopya E, Ndo C, Fossog BT, *et al.*: **Pyrethroid resistance and susceptibility to *Plasmodium falciparum* infection of *Anopheles coluzzii* populations from Yaoundé (Cameroon)**. *J Entomol Zool Stud.* 2015; **3**(6): 05–10. [Reference Source](#)
- Ndo C, Kopya E, Donbou MA, *et al.*: **Elevated *Plasmodium* infection rates and high pyrethroid resistance in major malaria vectors in a forested area of Cameroon highlight challenges of malaria control**. *Parasites Vectors.* 2018; **11**(1): 157. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Tchouakui M, Riveron JM, Djonabaye D, *et al.*: **Fitness Costs of the Glutathione S-Transferase Epsilon 2 (L119F-GSTe2) Mediated Metabolic Resistance to Insecticides in the Major African Malaria Vector *Anopheles funestus***. *Genes (Basel).* 2018; **9**(12): 645. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Tchouakui M, Chiang MC, Ndo C, *et al.*: **A marker of glutathione S-transferase-mediated resistance to insecticides is associated with higher *Plasmodium* infection in the African malaria vector *Anopheles funestus***. *Sci Rep.* 2019; **9**(1): 5772. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Tene Fossog B, Kopya E, Ndo C, *et al.*: **Water Quality and *Anopheles gambiae* Larval Tolerance to Pyrethroids in the Cities of Douala and Yaoundé (Cameroon)**. *J Trop Med.* 2012; **2012**: 429817. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

24. Antonio-Nkondjio C, Tene Fossog B, Kopya E, *et al.*: **Rapid evolution of pyrethroid resistance prevalence in *Anopheles gambiae* populations from the cities of Douala and Yaoundé (Cameroon).** *Malar J.* 2015; **14**: 155. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
25. Antonio-Nkondjio C, Poupardin R, Tene BF, *et al.*: **Investigation of mechanisms of bendiocarb resistance in *Anopheles gambiae* populations from the city of Yaoundé, Cameroon.** *Malar J.* 2016; **15**(1): 424. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
26. Doumbe-Belisse P, Ngadjieu CS, Sonhafouo-Chiana N, *et al.*: **High malaria transmission sustained by *Anopheles gambiae* s.l. occurring both indoors and outdoors in the city of Yaoundé, Cameroon [version 1; peer review: 2 approved].** *Wellcome Open Res.* 2018; **3**: 164. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
27. Talipouo A, Ngadjieu CS, Doumbe-Belisse P, *et al.*: **Malaria prevention in the city of Yaoundé: knowledge and practices of urban dwellers.** *Malar J.* 2019; **18**(1): 167. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
28. Antonio-Nkondjio C, Ndo C, Njiokou F, *et al.*: **Review of malaria situation in Cameroon: technical viewpoint on challenges and prospects for disease elimination.** *Parasit Vectors.* 2019; **12**(1): 501. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
29. Antonio-Nkondjio C, Fossog BT, Ndo C, *et al.*: ***Anopheles gambiae* distribution and insecticide resistance in the cities of Douala and Yaoundé (Cameroon): influence of urban agriculture and pollution.** *Malar J.* 2011; **10**: 154. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
30. Tene BF, Poupardin R, Costantini C, *et al.*: **Resistance to DDT in an urban setting: common mechanisms implicated in both M and S forms of *Anopheles gambiae*. in the city of Yaoundé Cameroon.** *PLoS one.* Public Library of Science; 2013; **8**(4): e61408. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
31. Livak KJ, Schmittgen TD: **Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta C_t}$ Method.** *Methods.* 2001; **25**(4): 402–8. [PubMed Abstract](#) | [Publisher Full Text](#)
32. Santolamazza F, Mancini E, Simard F, *et al.*: **Insertion polymorphisms of *SINE200* retrotransposons within speciation islands of *Anopheles gambiae* molecular forms.** *Malar J.* BioMed Central; 2008; **7**: 163. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
33. Bass C, Nikou D, Donnelly MJ, *et al.*: **Detection of knockdown resistance (*kdr*) mutations in *Anopheles gambiae*: a comparison of two new high-throughput assays with existing methods.** *Parasit Vectors.* 2007; **6**: 111. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
34. Christophe AN: **Nkahe *et al* 2020.** <http://www.doi.org/10.17605/OSF.IO/C8EUX>
35. Bamou R, Sonhafouo-Chiana N, Mavridis K, *et al.*: **Status of Insecticide Resistance and Its Mechanisms in *Anopheles gambiae* and *Anopheles coluzzii* Populations from Forest Settings in South Cameroon.** *Genes (Basel).* 2019; **10**(10): 741. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
36. Ferguson HM, Maire N, Takken W, *et al.*: **Selection of mosquito life-histories: a hidden weapon against malaria?** *Malar J.* BioMed Central; 2012; **11**: 106. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
37. Antonio-Nkondjio C, Youmsi-Goupeyou M, Kopya E, *et al.*: **Exposure to disinfectants (soap or hydrogen peroxide) increases tolerance to permethrin in *Anopheles gambiae* populations from the city of Yaoundé, Cameroon.** *Malar J.* Springer; 2014; **13**: 296. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
38. Briegel H, Hörler E: **Multiple blood meals as a reproductive strategy in *Anopheles* (Diptera: Culicidae).** *J Med Entomol.* Oxford University Press Oxford, UK; 1993; **30**(6): 975–985. [PubMed Abstract](#) | [Publisher Full Text](#)
39. Gimnig JE, Ombok M, Otieno S, *et al.*: **Density-dependent development of *Anopheles gambiae* (Diptera: Culicidae) larvae in artificial habitats.** *J Med Entomol.* Oxford University Press Oxford, UK; 2002; **39**(1): 162–172. [PubMed Abstract](#) | [Publisher Full Text](#)
40. Saingamsook J, Yanola J, Lumjuan N, *et al.*: **Investigation of Relative Development and Reproductivity Fitness Cost in Three Insecticide-Resistant Strains of *Aedes aegypti* from Thailand.** *Insects.* Multidisciplinary Digital Publishing Institute; 2019; **10**(9): 265. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
41. Brito LP, Linss JG, Lima-Camara TN, *et al.*: **Assessing the effects of *Aedes aegypti kdr* mutations on pyrethroid resistance and its fitness cost.** *PLoS One.* Public Library of Science; 2013; **8**(4): e60878. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
42. Foster SP, Young S, Williamson MS, *et al.*: **Analogous pleiotropic effects of insecticide resistance genotypes in peach-potato aphids and houseflies.** *Heredity (Edinb).* Nature Publishing Group; 2003; **91**(2): 98–106. [PubMed Abstract](#) | [Publisher Full Text](#)
43. Takken W, Smallegange RC, Vigneau AJ, *et al.*: **Larval nutrition differentially affects adult fitness and *Plasmodium* development in the malaria vectors *Anopheles gambiae* and *Anopheles stephensi*.** *Parasit Vectors.* BioMed Central; 2013; **6**(1): 345. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
44. Vantaux A, Lefèvre T, Cohuet A, *et al.*: **Larval nutritional stress affects vector life history traits and human malaria transmission.** *Sci Rep.* Nature Publishing Group; 2016; **6**: 36778. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
45. Carnevale P, Robert V, Manguin S, *et al.*: **Les anophèles: biologie, transmission du Plasmodium et lutte antivectorielle.** IRD. ed. Montpellier, France. 2009. [Reference Source](#)
46. Otali D, Novak RJ, Wan W, *et al.*: **Increased production of mitochondrial reactive oxygen species and reduced adult life span in an insecticide-resistant strain of *Anopheles gambiae*.** *Bull Entomol Res.* Cambridge University Press; 2014; **104**(3): 323–33. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
47. Tchakounte A, Tchouakui M, Mu-Chun C, *et al.*: **Exposure to the insecticide-treated bednet PermaNet 2.0 reduces the longevity of the wild African malaria vector *Anopheles funestus* but GSTE2-resistant mosquitoes live longer.** *PLoS One.* Public Library of Science; 2019; **14**(3): e0213949. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
48. Chabi J, Baidoo PK, Datsomor AK, *et al.*: **Insecticide susceptibility of natural populations of *Anopheles coluzzii* and *Anopheles gambiae* (*sensu stricto*) from Okyereko irrigation site, Ghana, West Africa.** *Parasit Vectors.* BioMed Central; 2016; **9**: 182. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
49. David JP, Strode C, Vontas J, *et al.*: **The *Anopheles gambiae* detoxification chip: a highly specific microarray to study metabolic-based insecticide resistance in malaria vectors.** *Proc Natl Acad Sci U S A.* National Acad Sciences; 2005; **102**(11): 4080–4084. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
50. Antonio-Nkondjio C, Ndo C, Njiokou F, *et al.*: **Review of malaria situation in Cameroon: technical viewpoint on challenges and prospects for disease elimination.** *Parasit Vectors.* 2019; **12**(1): 501. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Open Peer Review

Current Peer Review Status:   

Version 2

Reviewer Report 29 September 2020

<https://doi.org/10.21956/wellcomeopenres.17911.r40323>

© 2020 Edi A. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Ako Victorien Constant Edi

Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Cote d'Ivoire

The authors considered the comments and made the necessary corrections within the manuscript.

I have no further comments to make.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Vector biology, medical entomology and molecular biology and Epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 22 September 2020

<https://doi.org/10.21956/wellcomeopenres.17911.r40324>

© 2020 Afrane Y. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Yaw A. Afrane

Department of Medical Microbiology, College of Health Sciences, University of Ghana, Accra, Ghana

I still think this paper cannot be indexed because the life history traits of an insectary susceptible strain bred over 10 years cannot be compared with that of a colony from the field that has been selected with an insecticide for several generations. The comparison should have been made with another colony from the field which will not be selected by any insecticide that will lose the

resistance status over time. Insectary strain that has been kept for over 10 years have different life history traits such as fecundity, development time of the larvae, pupation rates etc. compared to a strain selected with an insecticide every other generation. Several studies have shown that resistant mosquitoes have a fitness cost (Alout *et al.*, 2014¹).

Therefore, what do the authors want to show by comparing that with a lab susceptible strain?

My issue with the paper is in the design of the experiment based on what I have raised even if "it doesn't make any sense" to the authors.

References

1. Alout H, Djègbè I, Chandre F, Djogbénou LS, et al.: Insecticide exposure impacts vector-parasite interactions in insecticide-resistant malaria vectors. *Proc Biol Sci.* 2014; **281** (1786). [PubMed Abstract](#) | [Publisher Full Text](#)

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Mosquito ecology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Version 1

Reviewer Report 02 September 2020

<https://doi.org/10.21956/wellcomeopenres.17594.r39620>

© 2020 Afrane Y. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Yaw A. Afrane 

Department of Medical Microbiology, College of Health Sciences, University of Ghana, Accra, Ghana

The authors are reporting their study on the "Fitness cost of insecticide resistance on the life-traits of a *Anopheles coluzzii* population from the city of Yaoundé, Cameroon". Insecticide resistance is the most important factor limiting the success of malaria control program in Africa. More data are needed to understand the effect of insecticide resistance on malaria mosquitoes; the fitness cost involved in getting resistance and how it affects their potential to transmit malaria and other vector-borne diseases. Therefore, the authors have chosen an important subject in malaria research to undertake their study.

However, enthusiasm is diminished by weaknesses in the design of the study, and to some extent,

the presentation of the results, making it difficult to recommend it for publication. Insectary strains of mosquitoes are known to have reduced resilience compared to wild caught mosquitoes. Being kept in captivity over several generations over the years makes the mosquitoes lose so many life-history traits, including resistant alleles, more especially as they are not under any selection pressure. Wild mosquitoes are under several pressures that could affect their malaria transmission potential. For a colony that has been kept for 14 years since 2006, their life history traits have been affected considerably compared to mosquitoes in the wild. Therefore, to make a comparison of wild-caught resistant mosquitoes that have been selected with deltamethrin for 12 generations with insectary colonized ones is not sound in my opinion. There should have been another wild-caught colony mosquitoes from the same area that were not selected with insecticide which would eventually lose their resistant abilities at some point to make the comparison. This colony would have been the unselected or susceptible strain. The insectary colony should have been a control strain for this experiment. Because of this weakness in the design of the experiment, I am unable to recommend it for publication.

Other comments are as follows:

- The manuscript needs a revision for its language to bring clarity to the text.

Methods:

- It is not clear if the same mosquitoes that were hatched from the blood feeding of the adults in the first instance, were the same mosquitoes that were reared to study their development time, pupation rate, emergence rate, and also for their adult longevity. If it is the same that was used then this should be clearly stated.

Results

- Under the heading "Fecundity and fertility" There is no information on the susceptible colony. Please compare the two colonies.
- Under the heading "Life span of the progeny of susceptible and resistant adults Mosquitoes" What was the difference between resistant and susceptible colonies for males and females. This was compared
- Under the heading "Molecular identification and *kdr*" The authors report that "molecular identification performed on field-collected mosquitoes (F0 generation) showed that 7.5% (7/93) belong to *An. gambiae* and 92.47% (86/93) were *An. coluzzii*." They go on to state that the F12 generation was all *An. coluzzii*. What happened to the *An. gambiae* population over time? Did they become fewer, got eliminated or their numbers were increased but were not part of the species ID done at the 12th generation. In addition, a better clarification of how many colonies involved in the study is needed in that section.
- Figure 2 legend needs revising. What actually is "B"? There is also no explanation to the figure called "Strata" in both Fig 2A and 2B

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Mosquito ecology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 04 Sep 2020

Christophe Antonio-N'kondjio, OCEAC, Yaoundé, Cameroon

Reviewer 3

Query 1: The authors are reporting their study on the "Fitness cost of insecticide resistance on the life-traits of a *Anopheles coluzzii* population from the city of Yaoundé, Cameroon". Insecticide resistance is the most important factor limiting the success of malaria control program in Africa. More data are needed to understand the effect of insecticide resistance on malaria mosquitoes; the fitness cost involved in getting resistance and how it affects their potential to transmit malaria and other vector-borne diseases. Therefore, the authors have chosen an important subject in malaria research to undertake their study. However, enthusiasm is diminished by weaknesses in the design of the study, and to some extent, the presentation of the results, making it difficult to recommend it for publication. Insectary strains of mosquitoes are known to have reduced resilience compared to wild caught mosquitoes. Being kept in captivity over several generations over the years makes the mosquitoes lose so many life-history traits, including resistant alleles, more especially as they are not under any selection pressure. Wild mosquitoes are under several pressures that could affect their malaria transmission potential. For a colony that has been kept for 14 years since 2006, their life history traits have been affected considerably compared to mosquitoes in the wild. Therefore, to make a comparison of wild-caught resistant mosquitoes that have been selected with deltamethrin for 12 generations with insectary colonized ones is not sound in my opinion. There should have been another wild-caught colony mosquitoes from the same area that were not selected with insecticide which would eventually lose their resistant abilities at some point to make the comparison. This colony

would have been the unselected or susceptible strain. The insectary colony should have been a control strain for this experiment.

Because of this weakness in the design of the experiment, I am unable to recommend it for publication.

Other comments are as follows:

- The manuscript needs a revision for its language to bring clarity to the text.

Response 1: We used a laboratory strain as control because it was not possible to have a field susceptible population. With the expansion of insecticide resistance in mosquito populations it has now become difficult to find susceptible field population in Yaoundé. The Ngouso strain used in this case as control originate from Yaoundé and is of the species *An. coluzzii* and is known for its high susceptibility to all insecticide classes. Several experimental study design comparing laboratory strains to field population have been reported in the literature and are still conducted nowadays. For instance the Kisumu laboratory strain is largely used for experimental studies and also in WHO bioassays to check the quality of impregnated papers. The argument that using a laboratory strain is a major weakness in the design of our study doesn't make sense we totally disagree with the reviewer's point of view.

Method

Query 2 : It is not clear if the same mosquitoes that were hatched from the blood feeding of the adults in the first instance, were the same mosquitoes that were reared to study their development time, pupation rate, emergence rate, and also for their adult longevity. If it is the same that was used then this should be clearly stated.

Response 2: The same mosquitoes were followed in the course of the study, please refer to the text and the raw data file for further details.

Results

Query 3: Under the heading "Fecundity and fertility" There is no information on the susceptible colony. Please compare the two colonies.

Response 3: The information is clearly presented in the section (please read through the section).

Query 4: Under the heading "Life span of the progeny of susceptible and resistant adults Mosquitoes" What was the difference between resistant and susceptible colonies for males and females. This was compared

Response 4: The information is provided in figure 2, please check graph B (and the legend above the graph for details).

Query 5: Under the heading "Molecular identification and *kdr*" the authors report that molecular identification performed on field-collected mosquitoes (F0 generation) showed that 7.5% (7/93) belong to *An. gambiae* and 92.47% (86/93) were *An. coluzzii*." They go on to state that the F12 generation was all *An. coluzzii*. What happened to the *An. gambiae* population over time? Did they become fewer, got eliminated or their numbers were increased but were not part of the species ID done at the 12th generation.

In addition, a better clarification of how many colonies involved in the study is needed in that section.

Response 5: PCR analysis of over 200 additional samples from the resistant colony (F12 generation) indicated that it was constituted of only *An. coluzzii* individuals.

Query 6: Figure 2 legend needs revising. What actually is "B"? There is also no explanation to the figure called "Strata" in both Fig 2A and 2B

Response 6: "B" refers to the second graph on the figure (the survival probability of male and female of the resistant and susceptible colonies). We provided additional details on the figure legend.

Competing Interests: No competing interests were disclosed.

Reviewer Report 21 August 2020

<https://doi.org/10.21956/wellcomeopenres.17594.r39619>

© 2020 Okumu F. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Fredros O. Okumu 

Environmental Health and Ecological Sciences Department, Ifakara Health Institute, Ifakara, Tanzania

The work by Nkahe *et al.* addresses an important question on how pyrethroid resistance might impact several life cycle traits of malaria vectors, and how this may translate to performance of control measures.

The writing is excellent, study methods properly described and the scientific rationale clearly stated.

I found it particularly interesting that susceptible mosquitoes live so much longer than resistant ones (~22 days compared to ~15 days). Coupled with the reduced blood-feeding proportions, this could mean very high levels of impact from insecticidal-interventions even if they do not impart direct mortality. It would be greater if this was discussed further with respect to the expected performance of interventions such as pyrethroid-treated nets.

Two minor comments are as follows:

- In the discussion section, the authors indicate that most of the fitness costs were in the aquatic stages, yet we see some great reductions on fecundity and adult survival as well. Since vector survival has far greater impacts on pathogen transmission than actual densities, it is perhaps incorrect to conclude that the fitness costs are mostly in the pre-adult stages.

- Would be great to see clarity on the decision to use 12 generations of the resistant colonies

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Public Health; Infectious Tropical Diseases; Vector Biology & Control

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 04 Sep 2020

Christophe Antonio-N'kondjio, OCEAC, Yaoundé, Cameroon

Reviewer 2

info_outline

The work by Nkahe *et al.* addresses an important question on how pyrethroid resistance might impact several life cycle traits of malaria vectors, and how this may translate to performance of control measures.

The writing is excellent, study methods properly described and the scientific rationale clearly stated.

Query 1: I found it particularly interesting that susceptible mosquitoes live so much longer than resistant ones (~22 days compared to ~15 days). Coupled with the reduced blood-feeding proportions, this could mean very high levels of impact from insecticidal-interventions even if they do not impact direct mortality. It would be greater if this was discussed further with respect to the expected performance of interventions such as pyrethroid-treated nets.

Response 1: The following phrase was added in the discussion section "Although the use of

pyrethroid treated nets are considered to induce a low mortality in resistant individuals, resistant mosquitoes were however found to exhibit reduce blood feeding rate, low fecundity and short adult survival rate all this somewhere suggest a long term impact of insecticide base intervention (such as pyrethroid treated nets) selection on vector population. This long term negative impact confirm continuous performance of pyrethroid treated nets interventions on vector populations. This unrecognized impact of treated nets need to be evaluated in different epidemiological settings”.

Two minor comments are as follows:

Query 2: In the discussion section, the authors indicate that most of the fitness costs were in the aquatic stages, yet we see some great reductions on fecundity and adult survival as well. Since vector survival has far greater impacts on pathogen transmission than actual densities, it is perhaps incorrect to conclude that the fitness costs are mostly in the pre-adult stages.

Response 2: The conclusion was corrected to address this comment.

Query 3: Would be great to see clarity on the decision to use 12 generations of the resistant colonies

Response 3: We selected mosquitoes for 12 generations to have highly resistant individuals and avoid susceptible individuals in the colony.

Competing Interests: No competing interests were disclosed.

Reviewer Report 21 August 2020

<https://doi.org/10.21956/wellcomeopenres.17594.r39850>

© 2020 Edi A. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Ako Victorien Constant Edi

Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Cote d'Ivoire

General comments:

- This study of Fitness cost of insecticide resistance on the life-traits of a *Anopheles coluzzii* population from the city of Yaoundé, Cameroon represents an important topic related to the insecticide resistance monitoring in the city of Yaoundé, Cameroon.
- Although resistance is largely expanding in *An. gambiae* s.l. populations from Cameroon, there has been little information so far on the influence of pyrethroid resistance on *An. gambiae* s.l. fitness and the current study generated data to support evidence.
- This study compared data from an insecticide resistant *An. coluzzii* colony from the city of Yaoundé, to a susceptible *An. coluzzii* laboratory colony “the Ngousso colony” to determine

life-traits parameters affected by the increased expansion of insecticide resistance in this vector population.

I recommend the current version of this paper for indexing with the very minor changes below to be considered by the authors.

Minor revisions:

- Key-words: *An. colluzzi* to be put in italics (*An. colluzzi*).

Introduction section:

- **In Line 3 to 5**, the sentence “**Four insecticide families, organophosphates, organochlorines, carbamates and pyrethroids, are used in public health**” needs to be corrected. With the introduction of SumiShield (neonicotinoid Clothianidin) to the market, the number of classes of insecticides has increased.

Methods section:

Study site:

- Line 13 to 15: Please add the reference to the following sentence: “**According to recent record it is estimated that over 75% of households in Yaoundé own at least a net**”.

Results section:

- Table 2. Differences in life-traits parameters between susceptible and resistant *Anopheles coluzzii*.
 - The Means \pm SE of resistant strain should be $93.33 \pm 10.77^*$ rather than $93.4 \pm 10.77^*$. Could you please check the writing of the numbers and choose if you want one or two numbers for the decimal and be constant throughout the manuscript.
 - Sex-ratio of adults mosquitoes:
 - In the sentence: “Of the 5226 mosquitoes who successfully emerged as adults in the susceptible group, 48.41% (N=2530) were males and 51.6% (N=2696) were females.”, the sum of both percentages is 100.1% instead of 100%, therefore it is important to choose either to keep 1 or (48.4% and 51.6%) or two numbers for the decimal (48.41% and 51.59%). Check throughout the manuscript.
 - In the sentence: “Of the 1907 mosquitoes who successfully emerged as adults in the resistant group, there was 51.49% (N=982) of males and 48.50% (N=925) females.”, the percentage should be 48.51% to have 100% when sum up both percentage values. Check throughout the manuscript.
 - Life span of the progeny of susceptible and resistant adults mosquitoes:
 - Line 10: In the sentence “The difference between the **average number** of males and females in each group was not significant for the susceptible group”, replace the average number by **average life span number**.

- Insecticide resistance profile of F0 and F12 generation:
 - What do the error bars mean on Figure 3 (confidence intervals or SEM?). Please add the meaning below the figure.
- Molecular identification and *kdr*:
 - Molecular identification performed on field-collected mosquitoes (F0 generation) showed that 7.5% (7/93) belong to *An. gambiae* and 92.47% (86/93) were *An. coluzzii*. While identification of 103 susceptible and 111 resistant (F12 generation) mosquitoes **confirms that the two groups belong to *An. coluzzii* species.** This finding is interesting and needs to be discussed in the Discussion section.
 - In Table 3: The sum of the percentage from N/119 is 101.53%, not 100%. This occurs because the percentage of RR should be 20.17% rather than 21.7%. Please check and correct throughout the manuscript.
 - In Table 3: The count of alleles (2N) should be 222 rather than 228 for the F12 generation ie. 2 x111. Please check and correct.
 - Please double check the number in each table and correct where necessary.

Discussion section:

- The authors with caution indicated that **“It is possible that in the present situation, the resistance status of *An. coluzzii* to insecticides also affects its vectorial competence. These findings still need to be validated by extensive field studies and experiments.”** Validation by further experimental studies will be important to improve knowledge on relation between vector competency and longevity.
- From F0, only *An. coluzzi* was selected over generation for female and male. Did the authors also find some *An. gambiae* s.s from emerging larvae across generations? If so what was the frequency relative to *An. coluzzii*?

Level of interest: An article of importance in its field.

Statistics: No statistical problem recorded.

Quality of written English: Acceptable.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Vector biology, medical entomology and molecular biology and Epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 04 Sep 2020

Christophe Antonio-N'kondjio, OCEAC, Yaoundé, Cameroon

Reviewer 1

General comments:

- This study of Fitness cost of insecticide resistance on the life-traits of a *Anopheles coluzzii* population from the city of Yaoundé, Cameroon represents an important topic related to the insecticide resistance monitoring in the city of Yaoundé, Cameroon.
- Although resistance is largely expanding in *An. gambiae* s.l. populations from Cameroon, there has been little information so far on the influence of pyrethroid resistance on *An. gambiae* s.l. fitness and the current study generated data to support evidence.
- This study compared data from an insecticide resistant *An. coluzzii* colony from the city of Yaoundé, to a susceptible *An. coluzzii* laboratory colony "the Ngousso colony" to determine life-traits parameters affected by the increased expansion of insecticide resistance in this vector population.

I recommend the current version of this paper for indexing with the very minor changes below to be considered by the authors.

Minor revisions:

Query 1: Key-words: *An. coluzzii* to be put in italics (*An. coluzzii*).

Response 1: Done.

Introduction section:

Query 2: In Line 3 to 5, the sentence "**Four insecticide families, organophosphates, organochlorines, carbamates and pyrethroids, are used in public health**" needs to be corrected. With the introduction of SumiShield (neonicotinoid Clothianidin) to the market,

the number of classes of insecticides has increased.

Response 2: Information added, see introduction.

Methods section:

Study site:

Query 3: Line 13 to 15: Please add the reference to the following sentence: “**According to recent record it is estimated that over 75% of households in Yaoundé own at least a net**”.

Response 3: Reference added.

Results section:

Table 2. Differences in life-traits parameters between susceptible and resistant *Anopheles coluzzii*.

Query 4: The Means \pm SE of resistant strain should be $93.33 \pm 10.77^*$ rather than $93.4 \pm 10.77^*$. Could you please check the writing of the numbers and choose if you want one or two numbers for the decimal and be constant throughout the manuscript.

Response 4: All changes done

Sex-ratio of adult mosquitoes:

Query 5: In the sentence: “Of the 5226 mosquitoes who successfully emerged as adults in the susceptible group, 48.41% (N=2530) were males and 51.59% (N=2696) were females.”, the sum of both percentages is 100.1% instead of 100%, therefore it is important to choose either to keep 1 or (48.4% and 51.6%) or two numbers for the decimal (48.41% and 51.59%). Check throughout the manuscript.

Response 5: Corrected and checking done throughout the manuscript.

Life span of the progeny of susceptible and resistant adults mosquitoes:

Query 6: Line 10: In the sentence “The difference between the **average number** of males and females in each group was not significant for the susceptible group”, replace the average number by **average life span number**.

Response 6: Changes done.

Insecticide resistance profile of F0 and F12 generation:

Query 7: What do the error bars mean on Figure 3 (confidence intervals or SEM?). Please add the meaning below the figure.

Response 7: The additional information was added below each figure

Figure 1: Error bars= Standard Deviation

Figure 3: Error bars= confidence intervals

Molecular identification and *kdr*:

Query 8: Molecular identification performed on field-collected mosquitoes (F0 generation) showed that 7.5% (7/93) belong to *An. gambiae* and 92.47% (86/93) were *An. coluzzii*. While identification of 103 susceptible and 111 resistant (F12 generation) mosquitoes **confirms that the two groups belong to *An. coluzzii* species**. This finding is interesting and needs to be discussed in the Discussion section.

Response 8: Only *An. coluzzii* was successfully maintained in laboratory across generations. The findings was discussed in the discussion section.

Query 9:

- In Table 3: The sum of the percentage from N/119 is 101.53%, not 100%. This occurs because the percentage of RR should be 20.17% rather than 21.7%. Please check and correct throughout the manuscript.
- In Table 3: The count of alleles (2N) should be 222 rather than 228 for the F12 generation ie. 2 x111. Please check and correct.

Response 9: Table 3 was corrected accordingly

Query 10: Please double check the number in each table and correct where necessary.

Response 10: Each number has been checked and corrected.

Discussion section:

- The authors with caution indicated that **“It is possible that in the present situation, the resistance status of *An. coluzzii* to insecticides also affects its vectorial competence. These findings still need to be validated by extensive field studies and experiments.”** Validation by further experimental studies will be important to improve knowledge on relation between vector competency and longevity.

Query 11: From F0, only *An. coluzzii* was selected over generation for female and male. Did the authors also find some *An. gambiae* s.s from emerging larvae across generations? If so what was the frequency relative to *An. coluzzii*?

Response 11: Apart from the f0 generation no *An. gambiae* larvae were found across generations. Over 200 additional specimens were processed and all turned to be *An. coluzzii*.

Competing Interests: No competing interests were disclosed.