Impact of sublethal pyrethroid exposure on resistant *Anopheles gambiae* mosquitoes' fitness

Alida Kropf
Universite de Neuchatel Institut de Biologie
Behi K Fodjo
Centre Suisse de Recherches Scientifiques en Cote d'Ivoire
Marius G Zoh
Laboratoire d'ecologie alpine
Chistabelle G Sadia
Centre Suisse de Recherches Scientifiques en Cote d'Ivoire
Affoué FP Kouadio
Centre Suisse de Recherches Scientifiques en Cote d'Ivoire
Chouaibou Mouhamadou (mouhamadou.chouaibou@csrs.ci)
CSRS, Cote d'Ivoire

Research

Keywords: Malaria, Vector, Insecticide, Resistance, Fitness

Posted Date: May 15th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-17262/v2

Abstract

Background : There is increasing evidence of insecticide resistance spreading among wild mosquito populations, which is widely believed to compromise vector control once it reaches a threshold that enables mosquitoes to survive exposure to long lasting treated bed-net (LLIN) or indoor residual spraying (IRS). However, very little is known about the long-term impact of insecticide resistance on malaria transmission, which makes the consequence of insecticide resistance spreading difficult to predict.

Methods: To gain more clarity, we have assessed five life-history traits of a resistant Anopheles gambiae laboratory strain that was repeatedly exposed to a LLIN and compared with individuals issued from the same strain but exposed to an untreated bed-net.

Results: The non-parametric Kruskal-Wallis test did not show any significant impact of gonotrophic cycle on the five traits. However, the Kolmogorov-Smirnov non-parametric test revealed a significant (i) drop in blood feeding mean rates ($D = 0.800$; $P< 0.0001$), (ii) increase in 24-hours post-exposure ($D = 0.600$; $P< 0.001$) and (iii) end of gonotrophic cycle mortality ($D = 0.611$; $P <0.006$), and (iv) drop in egg laying rate ($D = 0.730$, $P< 0.0001$) when mosquitoes were exposed. Surprisingly, there was rather an upward trend in the number of L3 larvae/female mosquito for the exposed group comparing to the unexposed one, although the difference was not significant ($D = 0.417$, $P> 0.05$).

Conclusion: Our study shows that in a context of widespread of resistance to insecticides, current pyrethroid-based vector control tools can still confer protection against malaria.

Introduction

Malaria is a parasitic disease that is transmitted to humans by female mosquitoes of the genus *Anopheles* [1]. It is considered as a major obstacle to development in Sub-Sahara African countries [2]. Large and sustained funding in the two last decade has enabled widespread geographical prevention of malaria disease. The most widely utilized strategies are the mass distribution of long lasting insecticidal nets (LLINs), indoor residual spraying (IRS) and artemisinin-based combination therapy (ACT). According to Bhatt et al, global malaria incidence has been reduced by 40% between 2000 and 2015 [3]. The authors estimate LLINs to be accountable for 68% of this reduction and ACT and IRS for 19% and 13%, respectively. For the period of 2016 to 2030, new goals and a new benchmark to reduce and eliminate malaria have been established taking into account the current situation [4-5]. The core methods of malaria control remain unchanged and rely on insecticides-based vector control. To date, only few classes of insecticides are approved by the World health organization (WHO) to be used in public health with only one, the class of pyrethroids, being recommended for LLINs [6]. Unfortunately, insecticide resistance is now widespread among malaria vector populations especially in sub-Saharan African regions [7-9]. Even more worrisome are reports from countries such as Côte d'Ivoire where resistance to all four classes of insecticides have been recorded [10] within the same mosquito populations. While much information has been gained on the biological and genetic mechanisms behind insecticide resistance, its

long term impact on vector control methods is poorly known and remains difficult to predict [11]. It is widely assumed that insecticide resistance will compromise vector control, as chemicals will no longer be able to kill upon contact [12-13]. As an evolutionary process, insecticide resistance has an associated fitness cost that can be reduced, for instance, through phenotypic plasticity. This associated cost has previously been assessed in regards to age, multiple exposures and delayed mortality [12,14-15]. The current study aims to investigate over the course of the mosquito's lifespan whether individuals that have survived insecticide exposure have a reduced fitness and reproductive success. This was assessed through the measurement after exposure to a sub-lethal dose of a public health insecticide of various life-history traits including blood-feeding rate, 24 hour post-exposure mortality rate, oviposition rate, mortality rate at the end of each gonotrophic cycle and the reproductive success. By assessing this, we hoped to gain a better understanding of insecticide resistance implication for malaria vector control.

Material And Methods

Mosquito strain

All experiments were conducted on a strain of *An. gambiae* isolated in 2016 from an *An. gambiae s.l.* strain, originating from the locality of Tiassalé in Côte d'Ivoire and bred without selection at the insectary of the *Centre Suisse de Recherches Scientifiques en Côte d'Ivoire* (CSRS) since 2010. According to WHO criteria [2, 16], Tiassale mosquito population has been described as resistant to at least three of the four classes of insecticides [17]. Both the *Kdr* and *Ace1R* point mutations, respectively responsible of resistance to pyrethroids and carbamates and organophosphates have been identified as a resistance mechanism in the *Tiassalé* strain, as well as metabolic resistance [10, 18]. The strain was a mixed of about 80% *An. coluzzii* and 20% *An. gambiae.* In order to isolate the *An. gambiae* from the original Tiassalé strain, pupae were isolated individually in plastic cups. After emergence, couples composed of a female and a male were formed and left for observation for five days to allow them to mate. A number was assigned to each couple. Following the oviposition, the parent couple was removed, killed and processed for molecular analysis through the Sine PCR [19] for species identification. Only larvae whose both parents were *An. gambiae* were kept and nurtured to adulthood. More than 200 couples were used to build up the new colony in order to reduce the effect of inbreeding. A subsample randomly selected in the F2 progeny was used to confirm the species status. The breeding conditions were standard insectary conditions of 27 ± 2 °C, 70 ± 5 % relative humidity (RH) and a 12 hours light:12 hours dark photoperiods. Eggs were placed in plastic trays of 15x30x5cm and filled with 800mL of dechlorinated tap water. Hatched larvae were separated into groups of about 200 larvae/tray in order to limit size variation. They were fed on mashed *Friskies* dry cat food. Starting day ten after hatching, pupae were collected for two days and transferred into 25x25x25cm holding cages for emergence. All emerged adults, before and after experimentation, were maintained under standard insectary conditions of 27 ± 2°C, 70 ± 5 % RH. They were provided with a 10% glucose solution daily. Experiments started with the F3 progeny.

Experimental design and processing

Prior the experimentation, a sub-lethal exposure time to deltamethrin treated bed-net that procured 20% was determined. The experimental design used has been drafted in order to mimic exposure to insecticides in natural settings where mosquitoes are coming in contact to the chemical on bed-net when they are trying to reach a person sleeping underneath for blood meal. Thus, a treated bed-net has been used. To increase accuracy, the bed-net was furnished by the National Control Program for Malaria (NCPM) of Côte d'Ivoire and was issued from the pool of Dawa 2.0 LLINs distributed to the population. It is coated with 80mg/m^2 of deltamethrin. Net Quality was assessed with a WHO standardized cone-test with the susceptible *An. gambiae* Kisumu strain maintained at the CSRS laboratory in Abidjan.

During the course of the experiment, four cohorts of 30 to 100 females aged four to five days depending on mosquito availability were separated into two same-sized groups. Each group was either exposed to a LLIN or to an untreated bed-net for 10 minutes, corresponding to the sub-lethal exposure time. For the exposure, mosquitoes were released into a 15X15X15cm cage that was covered with either a LLIN or an untreated net. The cage frames were wrapped with either treated fabric for the treated group or untreated fabric for the untreated group. After exposure, all individuals were transferred into a new holding cage according to their respective groups for 30 minutes recovery period whereafter, they were given the opportunity to have a blood meal for 30 minutes. All mosquitoes could rest and digest for two days before being transferred into individual 125 ml plastic cups. At this point, 24-hour mortality-post exposure and the blood feeding rates were recorded for each group. Each cup was lined at the bottom with damp cotton wool covered with a filter paper to stimulate oviposition. Each blood fed female was allowed to lay eggs for three days or until over 50% of the control group has laid. The egg laying rate or oviposition rate was defined as the number of females having laid at least one egg out of the total number of females that have taken a blood meal for each group. A second mortality was recorded at this point, corresponding to the mortality after a gonotrophic cycle. Afterwards, all females, fed and not fed, were transferred back into their initial cages and the experiment was repeated. Each cohort was exposed four times. Intervals between exposures were adapted to the times needed for egg laying.

Laid eggs were collected and pooled accordingly as exposed or non-exposed group and reared under standard insectary conditions. When larvae from each group grown to stage three (L3), they were counted and recorded. The Reproductive success was estimated as the mean number of L3 counted per female and per batch that had laid eggs.

Data analysis:

From the exposure to the end of gonotrophic cycle, four life-history traits were measured including the blood-feeding rate, the mortality rate 24-hour post-exposure, the mortality rate at the end of gonotrophic cycle, the egg laying rate and the reproductive success defined here as the mean number of L3 counted per female and per batch that had laid eggs.. Each trait was confronted to two independent variables, "gonotrophic cycle" and "exposure status" as well as their interaction. The non-parametric Kruskal-Wallis test with Bonferroni correction was used to test any significant difference between means of different traits among the variable "gonotrophic cycle", and the Kolmogorov-Smirnov non-parametric test was run

to compared the distribution of traits according to the variable “exposure status”. For both statistical methods, the level of significance was fixed at 5%. Statistical analysis was performed via XLSTAT version 2019.3.2 [20].

Results

A total of 477 females *An. gambiae* from the TIAS strain split into two groups were either exposed or not to a LLIN and allowed to complete four gonotrophic cycles. Both groups of mosquitoes were well balanced. Details on sample sizes, cohort composition over the different gonotrophic cycles are given in Table 1 as supplementary information. The unexposed mosquitoes consisted of 4 cohorts (or replicates) of 50, 100, 28 and 60 mosquitoes for a total of 238 versus 4 cohorts of 50, 100, 29 and 60 mosquitos for the exposed group for a total of 239 mosquitoes (Table 1, Additional information).

For both the unexposed and exposed groups, none of the five traits studied (including the blood-feeding rate, the mortality rate 24-hour post-exposure, the mortality rate at the end of gonotrophic cycle, the egg laying rate and the mean number of L3 counted per female and per batch that had laid eggs) appear to be significantly affected by the gonotrophic cycle within each group as their variation was not significant from gonotrophic cycles 1 to 4. However, these parameters were negatively affected once the mosquitoes were exposed to the treated net. Their variation were significant when the deltamethrin-exposed group was compared to unexposed group.

Regarding the blood-feeding, its rate was relatively stable between the different gonotrophic cycles in the unexposed group, and varied between 66.8% and 82.7%, the difference was not significant (P=0.909; Fig. 1a). In the exposed group, this parameter varied between 32.2% and 51.3% within the gonotrophic cycles. As in the unexposed group, the difference however was not significant (P= 0.351; Fig. 1a). But when compared the unexposed to the exposed group, a significant drop in blood feeding mean rates was observed (D = 0.800; P< 0.0001; Fig. 1b).

With regards to the mortality 24 hours post-exposure, we did not observed any significant variation between the gonotrophic cycles either in the unexposed group (5.9 to 11.9%; P= 0.847; Fig. 2a) or in the exposed group (11.1 to 38.9%; P= 0.071; Fig. 2a) despite that the trend was upward in the exposed group (Fig. 2a). However, when the two groups are compared together, the increase in mean mortality rate in the exposed group is significant (D = 0.600; P< 0.001; Fig. 2b).

Concerning the mortality at the end of the gonotrophic cycle or delayed mortality, we noted a non-significant variation in the unexposed group between the gonotrophic cycles (19.2 to 35%; P= 0.307; Fig. 3a) as well as in the exposed group (31.7 to 74.5%; P= 0.197; Fig. 3 a), despite that it was increasing over the gonotrophic cycles in the latter. However, when the two groups were compared together, the increase in the mortality at the end of gonotrophic cycle was significant (D = 0.611; P <0.006; Fig. 3b).

Like the blood-feeding rate or the mortality, the oviposition rate did not vary significantly between the gonotrophic cycles in the unexposed (33.3 to 21.4%; P= 0.841) nor in exposed group (15.0 to 9.6%;

P=0.667), although there was a slight downward trend in both groups (Fig. 4a). As for the other above traits, the egg laying rate was significantly affected by exposure to the treated net (D = 0.730, P< 0.0001, Fig. 4b).

As all for the four traits listed above, we did not find any significant variation in the reproductive success between the different gonotrophic cycles in both the unexposed group (P= 0.472) and the exposed group (P= 0.778;Fig. 5a). Contrary to what might have been expected in view of the results obtained on the other traits, the reproductive success did not decrease following exposure. Although it was not significantly different between the two groups (D = 0.417, P> 0.05, Fig. 5b), there was rather an upward trend in the exposed group comparing to the unexposed one.

Discussion And Conclusion

Resistance to insecticide has a certain fitness cost on the short and long-term that can be observed on a phenotypic level [21- 25] However, the extend to which this can maintain the sustainability of the species requires further investigation. Here, we assessed four energy-cost related life-history traits in resistant mosquitoes after a sublethal exposure to insecticide treated net and their epidemiological importance.

The first examined trait being the blood-feeding rate. Females that were exposed to deltamethrin fed less than females that were not exposed. Our findings were consistent with Glunt et al., [26] and contrary to what was observed by Tchakounte et al., [27] who showed that exposure of mosquitoes to PermaNet 2.0 deltamethrin-treated bednet presented no effect of on their blood feeding ability. From an epidemiological point of view, our findings can be translated into a reduction in transmission as the drop in the rate of mosquito bites also means less risk of infection with plasmodium. In areas of low- to moderate-coverage with impregnated mosquito nets, it is obvious that the irritant effect of pyrethroids repels mosquitoes from treated households to untreated households. However, according to our study, we can imagine that the prior contact of these mosquitoes with insecticides in treated dwellings should favor a limitation of bites of individuals in untreated dwellings [26]. Such beneficial effect should lead in a decrease of plasmodium-infected mosquito bites. This is probable one of the reasons which explains the beneficial effect of indoor residual sprayings (IRS) in areas of insecticide resistance in mosquito populations, where there is no physical barrier between the sleeper and the mosquito as is the case with mosquito bednets. Indeed, our study suggests that in such areas, despite that IRS can fail to kill mosquitoes because of their resistance to insecticides, simple prolonged contacts can be enough to cause a drop in host seeking behavior [26], and therefore a decrease in malaria transmission. Possible explanation is that insecticide exposure can have an effect on chemo-sensitivity, hence, exposed female may be less likely to detect physical and chemical cues from their prey. The success of blood feeding is doubly important as it affects not only pathogen transmission but also reproduction and thus population density and dynamic. In our study, in addition to the low rate in the bloodfeeding of exposed mosquitoes, we also noted among this group a low rate of egg laying compared to non-exposed mosquito group. This effect can be explained through a resources based trade-off between fecundity and survival [28] as the over-production of detoxifying enzymes triggered upon exposure to insecticide is probably associated with energetic cost,

which impacts the resource available for egg laying. Such a regulation would indicates a high adaptative cost related to insecticide resistance [29], which have been already demonstrated in previous studies [30-34]. The low rate of egg laying would logically be translated epidemiologically into a decrease in vector density and a thus decrease in transmission.

Reduction of vectors longevity is the target of current insecticide-based malaria control methods and insecticide resistance management strategies [33,35-37]. In our setting, the longevity was expressed by the mortality both at 24 hours post exposure, and at the end of the gonotrophic cycle. These two mortalities were affected by exposure to LLIN at each gonotrophic cycle contrary to observation made in Burjina Faso where following single and multiple exposures to a PermaNet 2.0 LLIN only one of the four mosquito populations tested showed evidence of delayed mortality [38].in our study, it increases by twice between the first and fourth gonotrophic cycles in unexposed mosquitoes, while it tripled over the same interval in the exposed group. By the end of gonotrophic cycle 4, mosquitoes were aged 25 to 28 days. At this point, 85% of the control population was eliminated and 97% of the treated group. Such an overall mortality rate approximates what can be observed in the field where only about 20% of the female live up to day 20 [39]. Those 13% difference in population survival are important for malaria transmission as only old female transmit the parasite. Indeed, under favorable conditions in tropical regions, the principal malaria *plasmodium falciparum* parasite complete its development cycle in 10-12 days in mosquitoes. Thus, from our results, repetitive exposure to a sub-lethal dose of deltamethrin through LLIN can still be efficient in controlling transmission in presence of pyrethroids resistance. This may partly explain why LLINs efficacy does not seem to have been curtailed despite the widespread of pyrethroids resistance in African malaria vectors [11, 40].

However, when looking at the reproductive success, although the number of offspring per female was not significantly different between the two groups, we observed high numbers of L3 larvae in the exposed group comparing to the unexposed group. Maybe this is a result of a resource-based trade-off for species perpetuation as final investment. Nevertheless if the same observation is made in natural settings, it can be considered as a counterbalance to the decrease in the vector population due to combination of high mortality, low blood intake rate and low egg laying rate following mosquitoes exposure to treated surfaces. Although this counterbalance is not enough to compensate for the losses caused by exposure to insecticides, it can nevertheless explain why despite the large-scale deployment of vector control strategies and/or insecticide resistance management, there is no eradication of mosquito populations despite a significant drop in their densities. This justifies the spread of resistance phenomena and the maintenance of malaria transmission despite decades of insecticide-based vector control activities. In this study, no evidence of differed impacts of insecticide exposure on the offspring viability was observed at larval stage. These effects were not further analyzed as we consider the variance acting on those parameters to be too high to draw conclusions.

While mortality/longevity remains one of the most sensitive parameter in vector control and thus malaria transmission, several other mosquito life traits that are negatively affected by exposure to insecticide for a sublethal time can reduce vector transmission potential to a certain extent, and when combined

together, the overall impact on can be even much higher, although we have not estimate the magnitude. This study reinforces the hypothesis that in the current context of resistance to insecticides, especially to pyrethroids, the existing toolbox for vector control can still confer protection against malaria. However, these findings should be considered with cautious as observations can vary the intensity of insecticide resistance, especially that the rapid and widely spread of insecticide resistance which threatens the insecticide-based vector control programs is problematic and highlights the urgent need to develop new strategies to mitigate these phenomena and preserve the efficacy of control interventions.

Abbreviations

ACT: Artemisinin Combined Therapy, CSRS*: Centre Suisse de Recherches Scientifiques en Côte d'Ivoire*, GC: Gonotrophic Cycle, IRS: Indoor Residual Spraying, L3: larvae stage three, LLINs: Long Lasting Insecticidal Nets, NMCP: National Malaria Control Program, RH: Relative Humidity, WHO: World Health Organization. BF: blood-feeding, M24: mortality 24-hour post-exposure, EggL: egg laying, MGC: mortality at the end of gonotrophic cycle, RS: reproductive success, E: Exposed, NE: Non exposed.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Wellcome Trust [103995]

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contributions

AK carried out the laboratory study and drafted the manuscript. BKF, GSC, AKFP and ZGM helped with the laboratory experiments. CSM conceived and initiated the study, ran data analysis and improved the manuscript. All authors have seen and approved that last draft.

Acknowledgments:

Prof. Hilary Ranson for editing and improving the manuscript. We also thank Dr. Florence Fournet for the scientific and educational support throughout the study and Dr Jasmina Saric for revising the manuscript.

References

1. Macdonald G. The epidemiology and control of malaria. 1957. (Oxford Univ. Press, 1957)
2. WHO| World Malaria Report 2016. WHO Available at: http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/. (Accessed: 9th May 2017).
3. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. *Nature*. 2015; 526:207–11.
4. Ranson H, Lissenden N. Insecticide resistance in African Anopheles mosquitoes: a worsening situation that needs urgent action to maintain malaria control. *Trends in parasitology*. 2016; 32:187–196.
5. WHO | Global Technical Strategy for Malaria 2016–2030. *WHO* Available at: http://www.who.int/malaria/publications/atoz/9789241564991/en/. (Accessed: 24th November 2017).
6. WHO | Malaria vector control and personal protection. *WHO* Available at: http://www.who.int/malaria/publications/atoz/who_trs_936/en/. (Accessed: 9th May 2017)
7. Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends in parasitology*. 2011;27:91–98.
8. Corbel, Vincent, and Raphael N'Guessan. "Distribution, mechanisms, impact and management of insecticide resistance in malaria vectors: a pragmatic review." Anopheles mosquitoes-New insights into malaria vectors. *IntechOpen*, 2013.
9. Djogbénou L, Pasteur N, Akogbéto M, Weill M, Chandre F. Insecticide resistance in the Anopheles gambiae complex in Benin: a nationwide survey. *Medical and veterinary entomology*. 2011; 25.3: 256-267.
10. Edi CV, Koudou BG, Jones CM, Weetman D, Ranson H. Multiple-insecticide resistance in Anopheles gambiae mosquitoes, Southern Cote d'Ivoire. *Emerging infectious diseases*. 2012;18:1508.
11. Viana M, Hughes A, Matthiopoulos J, Ranson H, Ferguson HM. Delayed mortality effects cut the malaria transmission potential of insecticide-resistant mosquitoes. *Proceedings of the National*

Academy of Sciences. 2016;113:8975–8980.

12. WHO Global plan for insecticide resistance management in malaria vectors. (*World Health Organization*, 2012).
13. Kelly-Hope L, Ranson H, Hemingway J. "Lessons from the past: managing insecticide resistance in malaria control and eradication programmes." *The Lancet infectious diseases.* 2008;8:387–389.
14. Glunt KD, Thomas MB, Read AF. The effects of age, exposure history and malaria infection on the susceptibility of Anopheles mosquitoes to low concentrations of pyrethroid. *PLoS ONE*. 2011;6.9.
15. Chouaibou MS, Chabi J, Bingham GV, Knox TB, N'Dri L, Kesse NB, et al. Increase in susceptibility to insecticides with aging of wild Anopheles gambiae mosquitoes from Côte d'Ivoire. *BMC infectious diseases*. 2012 ;12:214.
16. Bagi J, Grisales N, Corkill R, Morgan JC, N'Falé S, Brogdon WG, et al. When a discriminating dose assay is not enough: measuring the intensity of insecticide resistance in malaria vectors. *Malaria journal*. 2015;14:210.
17. Chouaibou, M. S. *et al.* Increase in susceptibility to insecticides with aging of wild Anopheles gambiae mosquitoes from Côte d'Ivoire. *BMC infectious diseases.* 12.1**,** (2012): 214.
18. Chouaïbou M, Zivanovic GB, Knox TB, Jamet HP, Bonfoh B. Synergist bioassays: A simple method for initial metabolic resistance investigation of field Anopheles gambiae s.l. populations. *Acta Tropica*. 2014; 130:108–11.
19. Santolamazza F, Mancini E, Simard F, et al.: Insertion polymorphisms of SINE200 retrotransposons within speciation islands of Anopheles gambiae molecular forms. *Malaria Journal*. 2008; 7(1): 163.
20. Addinsoft (2020). XLSTAT 2019.4.1 statistical and data analysis solution. New York, USA. https://www.xlstat.com
21. Garrett-Jones C. Prognosis for Interruption of Malaria Transmission Through Assessment of the Mosquito's Vectorial Capacity. *Nature*. 1964; 204:1173–5.
22. Djogbénou, L., Noel, V. & Agnew, P. Costs of insensitive acetylcholinesterase insecticide resistance for the malaria vector *Anopheles gambiae* homozygous for the G119S mutation. *Malaria Journal*. 2010; 9, 12.
23. Berticat, C., Boquien, G., Raymond, M. & Chevillon, C. Insecticide resistance genes induce a mating competition cost in Culex pipiens mosquitoes. *Genetics Research* 2002; 79, 41–47.
24. Brown, Z. S., Dickinson, K. L. & Kramer, R. A. Insecticide resistance and malaria vector control: the importance of fitness cost mechanisms in determining economically optimal control trajectories. *Journal of economic entomology*. 2013; 106, 366–374.
25. Alam M, Waqas Sumra M, Ahmad D, Shah RM, Binyameen M, Ali Shad S. Selection, Realized Heritability, and Fitness Cost Associated With Dimethoate Resistance in a Field Population of Culex quinquefasciatus (Diptera: Culicidae). *Journal of economic entomology*. 2017;110:1252–8.
26. Glunt KD, Coetzee M, Huijben S, Koffi AA, Lynch PA, N'Guessan R, et al. Empirical and theoretical investigation into the potential impacts of insecticide resistance on the effectiveness of insecticide-

treated bed-nets. *Evolutionary Applications*. 2018; 11:431–41.

27. Tchakounte A, Tchouakui M, Mu-Chun C, Tchapga W, Kopia E, Soh PT, 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.* 2019;14(3): e0213949
28. Stearns SC. The evolution of life history traits: a critique of the theory and a review of the data. *Annual review of ecology and systematics*. 1977;8:145–171.
29. Rivero A, Magaud A, Nicot A, Vézilier J. Energetic cost of insecticide resistance in Culex pipiens mosquitoes. *Journal of medical entomology*. 2011;48:694–700.
30. Assogba BS, Milesi P, Djogbénou LS, Berthomieu A, Makoundou P, Baba-Moussa LS, et al. The ace-1 locus is amplified in all resistant Anopheles gambiae mosquitoes: fitness consequences of homogeneous and heterogeneous duplications. *PLoS biology*. 2016;14 (12).
31. Assogba BS, Djogbénou LS, Milesi P, Berthomieu A, Perez J, Ayala D, et al. An ace-1 gene duplication resorbs the fitness cost associated with resistance in *Anopheles gambiae*, the main malaria mosquito. *Scientific reports*. 2015; 5, 14529.
32. Lenormand, T., Guillemaud, T., Bourguet, D. & Raymond, M. Appearance and sweep of a gene duplication: Adaptative response and potential for new functions in the mosquito Culex pipiens. *Evolution.* 1998; 52, 1705–1712.
33. Huijben S, Paaijmans KP. Putting evolution in elimination: Winning our ongoing battle with evolving malaria mosquitoes and parasites. *Evolutionary applications*. 2018;11:415–430.
34. Birget PLG, Koella JC. A genetic model of the effects of insecticide-treated bed-nets on the evolution of insecticide-resistance. *Evolution, medicine, and public health*. 2015; 205–15.
35. Govella NJ, Okumu FO, Killeen GF. Insecticide-treated nets can reduce malaria transmission by mosquitoes which feed outdoors. *The American journal of tropical medicine and hygiene*. 2010;82:415–419.
36. Read AF, Lynch PA, Thomas MB. How to make evolution-proof insecticides for malaria control. *PLoS biology*. 2009;*7*(4).
37. Koella JC, Lynch PA, Thomas MB, Read AF. Towards evolution-proof malaria control with insecticides. *Evolutionary applications*. 2009;2:469–480.

38 Hughes, A., Lissenden, N., Viana, M. *et al. Anopheles gambiae* populations from Burkina Faso show minimal delayed mortality after exposure to insecticide-treated nets. *Parasites Vectors*.2020;**13,** 17.

39. Garret-Jones C, Shidrawi GR. Malaria vectorial capacity of a population of Anopheles gambiae. *Bulletin of the World Health Organization*. 1969;40:531–45. 47.
40. Edi CV, Djogbénou L, Jenkins AM, Regna K, Muskavitch MAT, Poupardin R, et al. CYP6 P450 Enzymes and ACE-1 Duplication Produce Extreme and Multiple Insecticide Resistance in the Malaria Mosquito Anopheles gambiae. *PLOS Genetics*. 2014; 10(3).

Figures

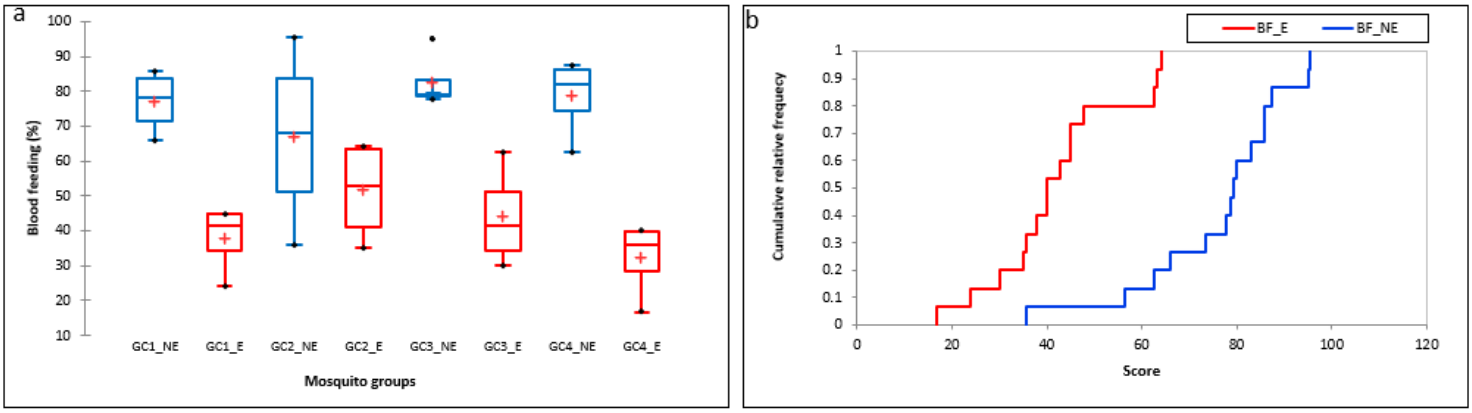

Figure 1

Blood-feeding of Anopheles gambiae after 30 minutes post-exposure at a sublethal time to a deltamethrin-treated long lasting bed-net. The boxplots represent the rates of blood feeding. On the box, the bottom line represents the lower quartile or 25% of individuals, the middle line is the median or 50% of individuals and the upper line is the upper quartile or 75% of individuals. The cross within the box is the mean value. Lines extending from the boxes are indicating variability outside the upper and lower quartiles. Individual points are outliers. The non-parametric Kruskal-Wallis test (a) did not show any significant difference between successive gonotrophic cycles (GC) 1 to 4 either in non-exposed (NE) mosquitoes (blue color) or in exposed (E) one (red color). However, the non-parametric Kolmogorov-Smirnov test revealed a significant drop in blood-feeding in the exposed group (b).

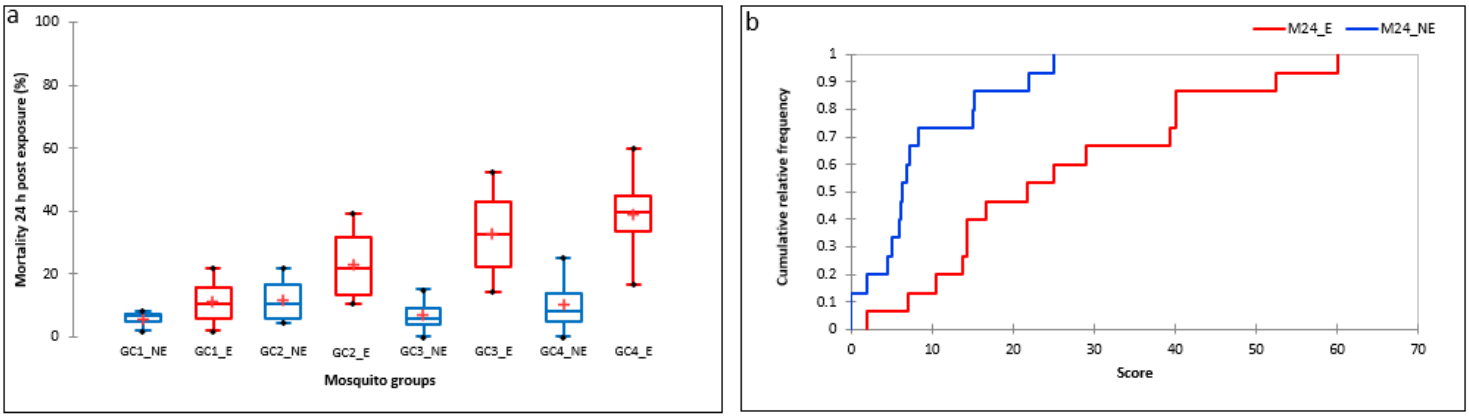

Figure 2

Mortality of Anopheles gambiae after 24 hours post-exposure at a sublethal time to deltamethrin-treated long lasting bed-net. The boxplots represent the rates of mortality. On the box, the bottom line represents the lower quartile or 25% of individuals, the middle line is the median or 50% of individuals and the upper line is the upper quartile or 75% of individuals. The cross within the box is the mean value. Lines extending from the boxes are indicating variability outside the upper and lower quartiles. The non-

parametric Kruskal-Wallis test (a) did not show any significant difference between successive gonotrophic cycles (GC) 1 to 4 either in non-exposed (NE) mosquitoes (blue color) or in exposed (E) one (red color). However, the non-parametric Kolmogorov-Smirnov test revealed a significant increase in mortality in the exposed group (b).

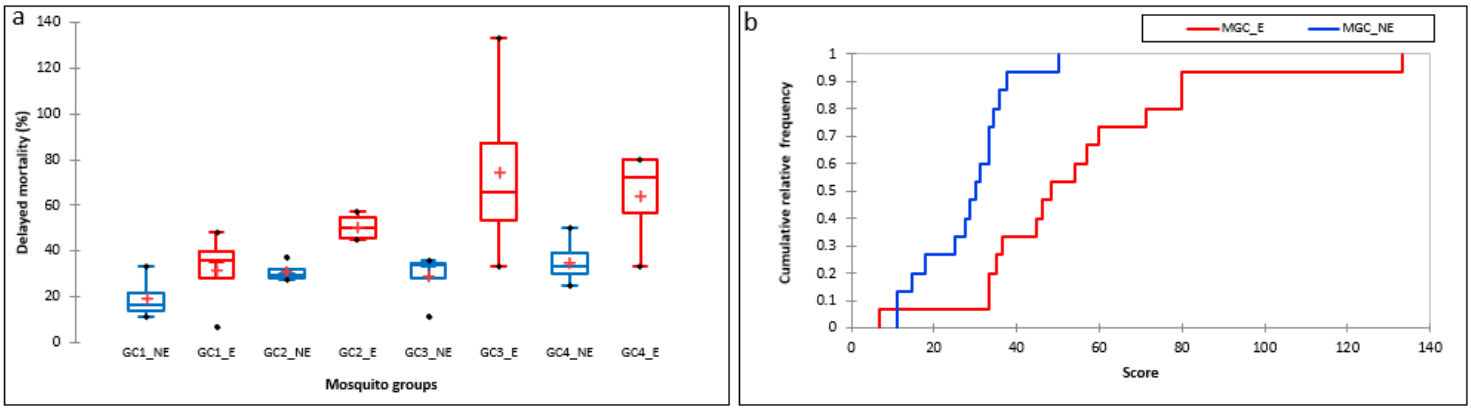

Figure 3

Mortality of Anopheles gambiae at the end of gonotrophic cycle (delayed mortality) following exposure at a sublethal time to deltamethrin-treated long lasting bed-net. The boxplots represent the rates of mortality. On the box, the bottom line represents the lower quartile or 25% of individuals, the middle line is the median or 50% of individuals and the upper line is the upper quartile or 75% of individuals. The cross within the box is the mean value. Lines extending from the boxes are indicating variability outside the upper and lower quartiles. Individual points are outliers. The non-parametric Kruskal-Wallis test (a) did not show any significant difference between successive gonotrophic cycles (GC) 1 to 4 either in non-exposed (NE) mosquitoes (blue color) or in exposed (E) one (red color). However, the non-parametric Kolmogorov-Smirnov test revealed a significant increase in mortality in the exposed group (b).

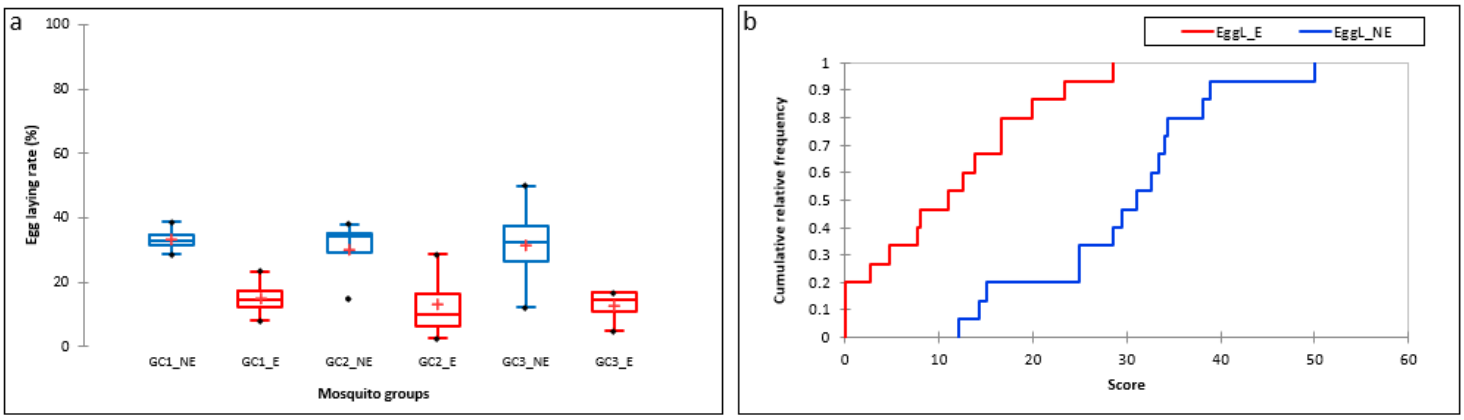

Figure 4

Egg laying rate of Anopheles gambiae following exposure at a sublethal time to deltamethrin-treated long lasting bed-net. The boxplots represent the rates of egg laying. On the box, the bottom line represents the lower quartile or 25% of individuals, the middle line is the median or 50% of individuals and the upper line

is the upper quartile or 75% of individuals. The cross within the box is the mean value. Lines extending from the boxes are indicating variability outside the upper and lower quartiles. Individual points are outliers. The non-parametric Kruskal-Wallis test (a) did not show any significant difference between successive gonotrophic cycles (GC) 1 to 3 either in non-exposed (NE) mosquitoes (blue color) or in exposed (E) one (red color). However, the non-parametric Kolmogorov-Smirnov test revealed a significant decrease in the rate of egg laying in the exposed group (b).

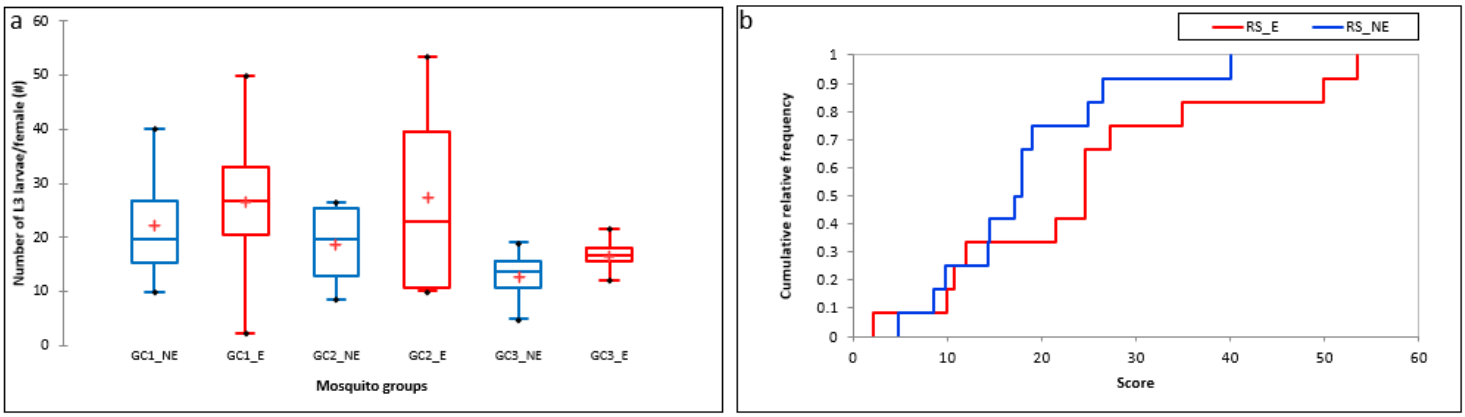

Figure 5

Reproductive success of Anopheles gambiae following exposure at a sublethal time to deltamethrin-treated long lasting bed-net. The boxplots represent the reproductive success, On the box, the bottom line represents the lower quartile or 25% of individuals, the middle line is the median or 50% of individuals and the upper line is the upper quartile or 75% of individuals. The cross within the box is the mean value. Lines extending from the boxes are indicating variability outside the upper and lower quartiles. The non-parametric Kruskal-Wallis test (a) did not show any significant difference between successive gonotrophic cycles (GC) 1 to 3 either in non-exposed (NE) mosquitoes (blue color) or in exposed (E) one (red color). However, there was an increase in the rate of egg laying in the exposed group (b).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Graphicalabstract.docx
- Additionalinformation.Table1.Cohortcomposition.xlsx