

Round #2

Author's Reply:

by *Stephanie Bedhomme*, 2020-10-13 08:55

Manuscript: <https://doi.org/10.1101/2020.06.08.139741> version
<https://doi.org/10.1101/2020.06.08.139741>

minor revisions

Dear authors,

The three reviewers and myself have acknowledge the changes made following the comments made on the first version of the manuscript and the four of us are convinced that this paper has to be published and recommended. However, before I recommend it, I would like the authors to make the wording changes strongly suggested by two of the reviewers. These changes are necessary to make the interpretation of your data and of the interest of the new method more cautious and realistic.

Stephanie Bedhomme

Additional requirements of the managing board:

As indicated in the 'How does it work?' section and in the code of conduct, please make sure that:

- Data are available to readers, either in the text or through an open data repository such as Zenodo (free), Dryad or some other institutional repository. Data must be reusable, thus metadata or accompanying text must carefully describe the data.
- Details on quantitative analyses (e.g., data treatment and statistical scripts in R, bioinformatic pipeline scripts, etc.) and details concerning simulations (scripts, codes) are available to readers in the text, as appendices, or through an open data repository, such as Zenodo, Dryad or some other institutional repository. The scripts or codes must be carefully described so that they can be reused.
- Details on experimental procedures are available to readers in the text or as appendices.
- Authors have no financial conflict of interest relating to the article. The article must contain a "Conflict of interest disclosure" paragraph before the reference section containing this sentence: "The authors of this preprint declare that they have no financial conflict of interest with the content of this article." If appropriate, this disclosure may be completed by a sentence indicating that some of the authors are PCI recommenders: "XXX is one of the PCI XXX recommenders."

Reviews

Reviewed by anonymous reviewer, 2020-10-02 16:13

The manuscript has been improved and for the most part, my concerns/ questions have been sufficiently addressed.

I do have one last comment about the wording describing the utility of the high-throughput field detection assay. In their response to reviewer comments, the authors explain that "the high-throughput diagnostic assay developed was not presented as a molecular assay allowing to track organophosphate resistance in this mosquito species but rather as a new tool to efficiently track this specific resistant mechanism." However in the manuscript, they are a bit less reserved about the implications of the assay: "will improve the tracking and management of organophosphate resistance in natural mosquito populations". If CCE amplification is not the dominant mechanism of organophosphate resistance in SEA, it may not be a useful assay for understand general resistance patterns. I assume that the frequency of this mechanism as opposed to others is not know and so I think the authors need to be more cautious in their statements about its utility for general resistance tracking and management.

> *This sentence has been modified as follows: "By reducing the human power and infrastructure needs associated with bioassays, this molecular assay will facilitate the tracking of organophosphate resistance alleles in natural Ae. aegypti populations."*

Reviewed by anonymous reviewer, 2020-09-28 13:17

This remains a nice paper that should be published. My comment about the strong interpretation and the claim of "confirmation" is one that I stand by (for reasons that I explain below), but an alternative to re-wording this term would be to at least argue against the possibility of drift in the manuscript (see below).

The authors' reply was the following:

"We agree that despite the large number of mosquitoes used for selection, our experimental selection design using a single replicate does not allow to fully control for genetic drift effects. However, the fact that this same CCE gene amplification was previously associated with organophosphate resistance in natural *Ae. aegypti* populations (see Faucon et al 2015, 2017, Poupardin et al 2014, Gouindin et al 2017, Marcombe et al 2009, ...) but also in *Aedes albopictus* (Grigoraki et al 2016 and 2017) clearly refutes the role of genetic drift in its increase frequency in our selected line. We agree that it would have been interesting to compare the frequency of this marker in dead and survivors of our laboratory lines following insecticide exposure but this would still not have constituted a proof of its role in resistance as a marker could have no functional role in resistance but still be segregated between dead and survivors as a consequence of being genetically linked with another resistance locus. A better way to perform such genotype-phenotype association study would be to use individuals or lines obtained from controlled crosses with a susceptible strain (at least F2) in order to break (by recombination) genetic links between markers and segregate individuals with different insecticide doses before investigating their CCE amplification genotype. While we such experiment will likely confirm the association of this marker with resistance, we thought that performing such heavy experiment was not necessary given the multiple evidences (including functional validation) supporting its role in organophosphate resistance. In conclusion, while we agree that our study does not constitute an irrefutable proof of the role of this CCE gene duplication in resistance we believe that the data we generated together with the published literature on this subject constitute enough evidence to support the role of this marker in organophosphate resistance in this mosquito species."

I disagree that previous evidence of the amplification being associated with organophosphate resistance in natural populations "refutes" the role of drift in their selected line, as the essence of the argument is circular: the known importance of the amplification is used to conclude that the results must be due to selection on the amplification, and these results are then used to claim "confirmation" of the importance of the amplification. If the importance of this amplification is so absolutely certain that it is unimaginable that it would not increase in frequency in the selected line, then the experiment has no value (we already knew the answer before it was conducted), the results add nothing to existing knowledge, and no "confirmation" is necessary. If on the other hand it was possible that selection would not lead to an increase in amplification frequency, then it is also feasible that the amplification could increase in frequency through drift. While the observed increase in frequency in a single replicate line with only two time points is indeed probably due to the importance of the amplification in resistance, it does not "confirm" it. While I would still prefer it if the word "confirm" were replaced with alternatives, such as "support" or "add weight to" or "are in accordance with", I think an alternative would be for the authors to instead add a few sentences arguing why the results are unlikely to be due to drift. For example, the increased expression in those same amplified genes that was seen after selection is unlikely to be selectively neutral, suggesting that drift is unlikely. Also, the fact that at least two independent amplification haplotypes have increased in frequency makes drift less likely. In either case, I would also change "confers" to "conferring" in the title, to remove the implication that the resistance association is a novel finding of this paper. I am happy for the editor to decide whether or not to recommend these changes.

> the term "confirms" was replaced by more cautious terms (i.e. "supports") throughout the whole manuscript. In addition, a sentence mentioning that the selection of these resistance alleles by genetic drift is unlikely was added to the discussion section as follows:

Line 665: "Although the potential role of genetic drift in the increased frequency of these resistance alleles in the selected Mala line cannot be fully excluded, their presence in organophosphate-resistant field populations makes it unlikely."

In regards of modifying the title of the manuscript, we would prefer to keep it as it is because changing "confers" for "conferring" will imply a complete rewording likely making it less clear for a broad audience. In addition, we believe that all changes made to the manuscript requested by reviewer 2 are now sufficient to clarify the added value of this work in regards of previous findings.

I would also like to revisit my comment about line 515 of the original manuscript (now line 549). My comment was:

line 515: "no false negative was observed" is redundant given the first part of this sentence. Also, could you comment on whether any false positives were observed?

and the reply from the authors was:

As stated in the manuscript, although the sybgrgreen qPCR assay slightly over-estimated copy numbers, all individuals identified as positive using this assay were also found positive using the TaqMan assay (no false positives). Reciprocally, all individuals identified as negative

using the sybgrgreen qPCR assay were also negative using the TaqMan assay (no false negatives).

The way this is phrased in the manuscript is still confusing in my opinion. The fact that "all individuals identified as positive using [the qPCR] assay were also found positive using the Taqman assay" is an indication that the Taqman assay had no false NEGATIVES (ie: no cases where the Taqman assay was negative when the qPCR gold standard was positive). On the other hand the fact that "all individuals identified as negative using the sybgrgreen qPCR assay were also negative using the TaqMan assay" is an indication that the Taqman assay had no false POSITIVES (no cases where the Taqman assay was positive when it should have been negative). Thus, the way that the manuscript is currently phrased is saying twice that there were no false negatives, and is not currently saying that there were no false positives.

> *This is now corrected in the manuscript*

Minor points and typos:

lines 51-52: In response to my previous comment, the authors explained what they mean here by "their usefulness", but the sentence in the manuscript remains unchanged and, in my opinion, unclear. First, it is not grammatically clear whether "their" refers to insecticides or amplifications. The authors replied to clarify that it referred to amplifications, but have not made the text clearer. Also, the meaning of the statement in the manuscript is odd: it is basically saying that resistance alleles (amplifications in this case) are useful for monitoring resistance alleles. Again, the authors explained what they meant in their reply, but the sentence in the manuscript is no less confusing than before.

> *The sentence in the abstract has been modified as follows: "In insects, the use of insecticides can select gene amplifications causing an increased expression of detoxification enzymes, supporting the usefulness of these DNA markers for monitoring the dynamics of resistance alleles in the field." The last sentence of the introduction (Line 125) has also been modified as well as those from Lines 713-718 and 763-764.*

line 54: developing -> to develop

> *Done*

line 75: Either change "such" to "this" or make "mechanism" plural (such mechanisms have been shown to be major drivers...)

> *Done*

line 86: "later" -> "latter"

> *Done*

line 111: such tool -> such a tool

> *Done*

line 360: The authors say that they have modified this sentence to remove the word "invalidating", but the sentence has remained unchanged.

>Done

line 438: This has been clarified by the authors in their reply, but remains unclear in the manuscript. It still comes across that 33% of G5-Mala individuals have haplotype A and 67% of G5-Mala individuals have haplotype B. In fact, as clarified by the authors in their reply, it is 33% of the amplified haplotypes that were A and 67% that were B. I think it would be clearer if the authors included exact numbers, as they did in their reply, or rephrased this to make it clear that 33% and 67% are not percentages of the G5-Mala population. Also, since it seems that the B haplotype cannot be detected in an AB heterozygote (because the presence of A masks the presence of B), I suggest clarifying that the that 67% is a minimum value (some of the 33% that have A might also have had B).

> *This has been clarified in the manuscript.*

line 577: Change "aiming at preventing" to either "aiming to prevent" or "aimed at preventing"

>Done

line 683: increase of the frequency -> increase in the frequency

>Done

line 684: comma after "selection"

>Done

Reviewed by Diego Ayala, 2020-10-11 15:44

Dear Editor,

I acknowledge the effort carried out by the authors in this new version. I have carefully gone through the manuscript' changes and answers to the reviewers and editor and I have been impressed by the quality of the details. The editor has arisen an important issue about the possibility that other genes left aside in the present manuscript could also be involved in the resistant phenotype. Although it is a potential caveat that it has to be considered, I have to say that I agree with the more conservative approach assumed by the authors and their explanations.

The manuscript will help to better understand the insecticide resistance in this major vector and will help its evolution across the world.

> *We thank Reviewer 3 for acknowledging all the changes made to the manuscript and supporting its recommendation by PCI Evolutionary Biology.*