Peer Community In Evolutionary Biology

A duplication driving metabolic insecticide resistance in *Aedes aegypti*

Diego A. Hartasánchez based on peer reviews by **Diego Ayala** and 1 anonymous reviewer

Tiphaine Bacot, Chloe Haberkorn, Joseph Guilliet, Julien Cattel, Mary Kefi, Louis Nadalin, Jonathan Filee, Frederic Boyer, Thierry Gaude, Frederic Laporte, Jordan Tutagata, John Vontas, Isabelle Dusfour, Jean-Marc Bonneville, Jean-Philippe David (2024) A genomic duplication spanning multiple P450s contributes to insecticide resistance in the dengue mosquito *Aedes aegypti*. bioRxiv, ver. 5, peer-reviewed and recommended by Peer Community in Evolutionary Biology. https://doi.org/10.1101/2024.04.03.587871

Submitted: 10 April 2024, Recommended: 18 November 2024

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Insecticide resistance in mosquitoes represents a notable challenge to public health efforts aimed at controlling vector-borne diseases. Among mosquito species, *Aedes aegypti* is particularly significant due to its extensive geographic spread and its ability to transmit arboviruses causing diseases such as dengue, yellow fever, Zika, and chikungunya (Brown et al., 2014). Insecticide resistance typically develops through two main mechanisms: target-site mutations, which affect the insecticide's interaction with its target, and metabolic resistance, in which insecticide detoxification is enhanced in mosquitoes. While target-site mutations are well characterized, the mechanisms underlying metabolic resistance are understudied.

The study by Bacot and colleagues (2024) contributes to our understanding of the genetic and evolutionary mechanisms driving insecticide resistance, focusing on a case of metabolic resistance in *Aedes aegypti* from French Guiana. Following the recent identification of a copy number variant region on chromosome 1, potentially linked to overexpression of detoxification enzymes (Cattel et al., 2020), this study explores the region's genomic architecture, its likely origin and provides compelling evidence for its role in insecticide resistance.

Through RNA sequencing and whole-genome pool sequencing, the authors reveal that this 220 kilobase duplication increases the expression level of several clustered P450 genes. Cytochrome P450s are known to play a role in breaking down pyrethroids like deltamethrin, a commonly used insecticide. The role of P450 enzymes in detoxification was demonstrated by treating mosquitoes with piperonyl butoxide, a P450 enzyme inhibitor, and observing reduction in deltamethrin resistance, further confirmed by RNA interference experiments. Despite the clear advantages of this genomic duplication in conferring resistance, the study

also uncovers a fitness cost associated with carrying the duplication. Through experimental evolution, the authors find that mosquitoes with the duplication experience reduced fitness in the absence of insecticide pressure. Given the regions structural complexity, the authors could not completely disassociate the effect of the duplicated region and that of a target-site mutation. However, they developed an assay that can accurately track the presence of this resistance allele in mosquito populations.

Altogether, the study by Bacot et al. (2024) highlights the challenges of characterizing the phenotypic effect of copy number variant regions in complex genomes, such as that of *Aedes aegypti*. It emphasizes the need for further studies on the origin and spread of this duplication to better understand how similar resistance mechanisms might evolve and disseminate. Overall, the completeness and coherence of the narrative, the detailed and thorough analysis, and the insightful discussion, make this work not only a significant contribution to the field of insecticide resistance but an interesting read for the general evolutionary biology community.

References:

Brown, J. E., Evans, B. R., Zheng, W., Obas, V., Barrera-Martinez, L., Egizi, A., Zhao, H., Caccone, A., & Powell, J. R. (2014). Human impacts have shaped historical and recent evolution in *Aedes aegypti*, the dengue and yellow fever mosquito. Evolution, 68(2), 514–525. https://doi.org/10.1111/evo.12281

Cattel, J., Faucon, F., Le Péron, B., Sherpa, S., Monchal, M., Grillet, L., Gaude, T., Laporte, F., Dusfour, I., Reynaud, S., & David, J. P. (2019). Combining genetic crosses and pool targeted DNA-seq for untangling genomic variations associated with resistance to multiple insecticides in the mosquito *Aedes aegypti*. Evolutionary applications, 13(2), 303–317. https://doi.org/10.1111/eva.12867

Tiphaine Bacot, Chloe Haberkorn, Joseph Guilliet, Julien Cattel, Mary Kefi, Louis Nadalin, Jonathan Filee, Frederic Boyer, Thierry Gaude, Frederic Laporte, Jordan Tutagata, John Vontas, Isabelle Dusfour, Jean-Marc Bonneville, Jean-Philippe David (2024) A genomic duplication spanning multiple P450s contributes to insecticide resistance in the dengue mosquito *Aedes aegypti*. bioRxiv, ver.5 peer-reviewed and recommended by PCI Evol Biol https://doi.org/10.1101/2024.04.03.587871

Reviews

Evaluation round #3

DOI or URL of the preprint: https://doi.org/10.1101/2024.04.03.587871
Version of the preprint: 4

Authors' reply, 12 November 2024

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Decision by Diego A. Hartasánchez, posted 08 November 2024, validated 10 November 2024

Dear Jean-Philippe David, Tiphaine Bacot and co-authors,

Thank you very much for submitting the revised version of your manuscript to PCI Evolutionary Biology. I consider that the reviewers' considerations have all been adequately addressed and I am now almost ready to proceed with my recommendation. There are two additional minor issues which would need to be addressed beforehand.

First, the manuscript would need to undergo a careful reading to correct spelling and grammar mistakes, style consistencies and other minor issues. I am sorry for not pointing these out before but it has only been upon careful re-reading of your manuscript that I detected them. Please find below a list of these and do try to be exhaustive since there are probably a few more which I did not detect myself.

Second, I would advise to deposit the Supplementary Material in a public repository such as Zenodo which allows to include descriptions such as the ones now included in the text. Please add the permanent doi identifier in the Data Availability section. Additionally, although the Materials and Methods section is quite exhaustive, I would advise to also provide the code used to conduct the statistical tests and, if possible, to produce the plots. Given the type of manuscript, I do not consider this to be absolutely necessary but in the spirit of reproducible science, I would strongly advise you to upload the code to a public repository and to include the permanent doi identifier in the Data Availability section.

I am sorry for not mentioning these two issues before and that the review process has taken so long. I will proceed with my recommendation once these issues have been addressed in a revised version.

Best regards,
Diego A. Hartasánchez

Minor issues

Line 25: extra “and”

Line 100: please try to be consistent throughout the text in the use of mathematical signs and units. There is sometimes a space after “>”, and sometimes there is none (e.g. line 631). There is sometimes a space between number and unit (e.g. line 667) and sometimes there is none (e.g. line 668).

Line 104: please be consistent with the use of “kdr” vs. “Kdr” mutations

Line 105 and 372: there appears to be a double space. Please check the entire manuscript.

Line 119: “reverse genetics”?

Line 128: “insectaries”?

Line 145: I would suggest describing the 20% increase by saying from x% to y% to avoid the potential misunderstanding that the value increased in 20% of the previous value (i.e. from 5% to 6%)

Line 145: please be consistent with the use of “p-value” vs. “P value”

Line 196: please use “vs.” instead of “Vs” for “versus” throughout the text and be consistent with the use of italics

Line 180 and 206: “fold” instead of “folds”. Please be consistent with the use of “2 fold increase” vs. “2-fold increase” as in line 267

Line 229: “expresses”

Line 298: please avoid collapsing the text onto Figure 4. Also, please explain what “a”, “b”, and “c” mean in Figure 4 top right

Line 310: “genes”

Line 351: “led”

Line 379: “through”

Line 358: “was”

Line 514: “Eppendorf”

Line 574: “manufacturer” or “manufacturer’s”

Line 587: 3’. I checked with several pdf viewers and I see a question mark inside a box symbol instead of the ’.

Line 609: “Oct.”
Line 659: “et al.,”. Please check for consistency across the text.
Line 687: “Acknowledgements”
Line 696: “Author” or “Authors”’
References: As for the case of 3’, there are multiple instances of this question mark sign, in particular (but not only) for the “-” in between page numbers. Potentially this could be solved by replacing “–” by “-”. Please check all references.

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/2024.04.03.587871
Version of the preprint: 3

Authors’ reply, 07 November 2024

Download author’s reply

Decision by Diego A. Hartasánchez, posted 29 October 2024, validated 31 October 2024

Dear Jean-Philippe David, Tiphaine Bacot and co-authors,

Thank you very much for submitting the revised version of your manuscript to PCI Evolutionary Biology. I have now received very favorable comments from two reviewers. They both consider that all of their comments on the first version have been addressed and are pleased with the current version.

There are just four minor comments pointed out by one of the reviewers. Once these are addressed I will gladly proceed with my recommendation.

I am very sorry that we have taken so long to complete this round of review. The recommendation will be published as soon as possible once your revised version is submitted.

Best regards,
Diego A. Hartasánchez

Reviewed by Diego Ayala, 29 October 2024

Dear Editor,
I have reviewed the revision of the Manuscript by Bacot et al.,. In the present version, the authors have carried out an extensive revision of the manuscript, including all the remarks and comments done by both reviewers. To my understanding, this new version has gained in clarity and precision. I congratulate to the authors for their work and recommend for its publication
Just few comments :
Line 35. Please include the paper Kamdem et al., 2017 MBE, which as a similar approach in Anopheles
Line 126. According to WHO, the IR13 should be considered as ”tolerant” and not as sensible?
Line 134. Please provide the dose
LIne 221-222. Do you mean that the resistant coply is fixed in the Ile-Met ?

Reviewed by anonymous reviewer 1, 18 September 2024

Dear,

The authors adequatly respond to our comments and we really appreciated reading the new version of the manuscript.

All the best,

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2024.04.03.587871

Version of the preprint: 2

Authors' reply, 03 September 2024

please see attached pdf file for our revised cover letter and responses to reviewers. see also attached .docx file for the track change revised version of the manuscript deposited on bioRxiv as V3 version.

Download author's reply

Download tracked changes file

Decision by Diego A. Hartasánchez, posted 25 June 2024, validated 25 June 2024

Major revision suggested

Dear Tiphaine Bacot and co-authors,

First of all, thanks for submitting your work to PCI Evolutionary Biology, and please forgive the delay in getting back to you with the reviews for your manuscript.

I enjoyed reading your manuscript and find that it's a research of great importance. I have received two reviews by experts in the field, which are also optimistic about your work. The reviewer's comments are mostly positive with respect to the importance of your work, and the quality of your manuscript, acknowledging the complications in performing this type of research in a challenging genome. They do, however, raise important points that would need to be addressed prior to my recommendation.

Please, address all of the reviewers' points. In particular, the findings of Cattel et al., 2020, should be encompassed adequately in the manuscript; apparent inconsistencies between figure 1 and table 1, and between figures 1 and 4, need to be clarified; and I do suggest reevaluating the substructure of the results section to see if you find a way to present the results in a way that helps clarify some of the unclear points of the manuscript.

Finally, although I consider that all of the reviewers comments and questions should be addressable, please feel free to explain your point of view if you happen not to agree with them.

I'm looking forward to your revisions.

Best regards,

Diego A. Hartasánchez

Reviewed by Diego Ayala, 20 June 2024

The manuscript by Bacot et al., presents a thorough and well-articulated study on the characterization of a genomic duplication that implement insecticide resistance in Aedes aegypti, a major vector of arbovirus across the World. The research is meticulously conducted, with a large number of different experiments and testing-hypothesis, with a clear exposition of methodologies and results. The authors look at to demonstrate the functional implications of the genomic duplication, supported by robust experimental data. The discussion provides insightful interpretations and situates the findings within the broader context of insecticide resistance mechanisms in this mosquito. It is clear that other similar genomic characterization of this duplication would help to udnerstsand its origin and spread. Overall, the clarity and coherence of the narrative, combined with the significance of the research, make this article a valuable contribution to the field and worthy of publication.

My unique concern is about how the authors integrate the previous results (Cattel et al., 2020) in the present study. The duplication was already observed and roughly characterized. However, even if they mention this fact in the introduction (i.e. line 97), they perform some analysis as if they knew little about the duplication. For instance, I wondered why they performed comparative analysis on F1 and F2 (lines 144 and so on) to understand if there is any maternal effect. It is now obvious when you see later that this duplication is in the chromosome 1. Moreover, they already knew that the duplication affected multiple P450s, however it seems as they discovered that in the paper. In my opinion, if the authors provide former information about their previous results, the paper will be easier to follow up.

A second, but minor question, is about the role of the duplication contributing to insecticide resistance. The article provides a comprehensive analysis of genomic duplication conferring insecticide resistance but occasionally presents controversial results regarding the duplication's role (i.e. lines 347-348, line 401). Clarifying the transitions between the results would enhance the manuscript's overall clarity.

Overall, the paper is very complete and of a high scientific level, with numerous assays spanning from phenotype to genotype. Congratulations to the authors for their meticulous work and significant contributions to the field of insecticide resistance research in general, and in Aedes aegypti in particular.

Download the review