RESPONSE LETTER TO THE COMMENTS OF THE RECOMMENDER AND REVIEWERS

Round 2

Resubmission MS Title:

Unraveling genetic load dynamics during biological invasion: insights from two invasive insect species

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Authors:

Eric Lombaert, Aurélie Blin, Barbara Porro, Thomas Guillemaud, Julio S. Bernal, Gary Chang, Natalia Kirichenko, Thomas W. Sappington, Stefan Toepfer, Emeline Deleury

Submitted to: PCI Evolutionary Biology

Dear Recommender,

Thank you for your positive feedback and for handling our manuscript throughout the review process. We have greatly appreciated the opportunity to improve our work based on the reviewers' insightful comments.

We have addressed the final minor points raised by Reviewer 1. Please find our detailed responses below (in italic characters).

We sincerely appreciate your time and consideration, and we look forward to your final decision.

Best wishes,

Eric Lombaert on behalf of all authors

Reviewer #1’s comments

For this revised version, the authors made several changes and performed additional analyses that strengthen the robustness of the results. They also clarified some methodological points and what was already known on the history of the two species. The discussion is also more balanced. So I think that this revised version is suitable for publication.

Thank you for your thoughtful comments throughout the review process. We greatly appreciate your feedback, which has helped us clarify key methodological points and strengthen the robustness of our results.

I just have a few additional minor comments.

About SNP polarization.

- I now understand the idea of using several pairs and to combine them to have a more robust polarization while in the first version I understood that each population had its own set of polarized SNPs. This is clearer now.

- Given the additional analysis on the effect of the threshold value (showing that there are almost no effect) I think the results are robust. However, weighting (or sampling) ancestral states as a function of probabilities is not a new idea. It is one of the interests of such an approach. For example, in Keightley PD, Campos JL, Booker TR, Charlesworth B. 2016. Inferring the Frequency Spectrum of Derived Variants to Quantify Adaptive Molecular Evolution in Protein-Coding Genes of Drosophila melanogaster. Genetics 203: 975–984. [Equation 4]

Thank you for pointing out this aspect of ancestral state weighting. In Keightley et al. (2016), this approach is used to improve the accuracy of unfolded site frequency spectrum estimation by addressing uncertainties in ancestral versus derived allele classification, thereby mitigating biases linked to substitution rate variation and selective constraints across sites. Notably, this method is also implemented in the software est-sfs, which we used in our study.

We fully acknowledge the relevance of the weighting approach you previously suggested and agree that it is a valuable method. However, as you also noted, we believe that our chosen approach remains robust and conservative. The distribution of probabilities we included in the previously revised manuscript strongly supports the absence of bias in our case.

We greatly appreciate this discussion, as it highlights complementary strategies for addressing ancestral state uncertainty.

L85: “Conversely…” Actually, the purging effect if the other side of the same process but on a slightly different time scale: Drift can expose deleterious alleles in homozygotes which can both reduce mean fitness and can lead to extinction but if the population escapes extinction, a part of the load has been purged. So, you may consider rephrase a bit this sentence. (See also the comment of reviewer 2 on the time for purging to be effective, compared to the direct fitness effect of exposing deleterious alleles in homozygotes).

We fully agree that the exposure of deleterious alleles due to drift and their subsequent removal by purifying selection are interconnected processes operating on different timescales. We have reworded the relevant passage to ensure that it does not imply an immediate purging effect at the onset of an invasion and to better distinguish between the short-term fitness consequences of increased homozygosity and the potential for genetic load reduction over multiple generations. The revised text also better aligns with Reviewer 2’s previous comments. See Lines 85-89: “Conversely, the increased homozygosity resulting from bottlenecks exposes recessive deleterious alleles to selection. In the short term, this exposure can reduce mean fitness, but if the population persists, purifying selection may progressively remove some highly deleterious alleles over multiple generations, potentially reducing genetic load (Dussex et al. 2023).”

Anonymous reviewer #2’s comments

Dear Editor and Authors,

I have now read the updated revision of Lombaert et al. entitle 'Unraveling genetic load dynamics during biological invasion: insights from two invasive insect species'.

I am glad to read that my comments were useful. I have now read the responses to my comments and changes they made to their manuscript. I feel that my concerns were addressed appropriately and that the changes made improved the quality of the manuscript.

Thank you very much for your positive feedback. We are glad that our revisions addressed your concerns and that you find the manuscript improved. We appreciate your helpful comments throughout the review process.