Peer Community In
Evolutionary Biology

The genetic load of invasive population: how little do we know ?

Quentin Rougemont *based on peer reviews by* ***Sylvain Glémin*** *and 2 anonymous reviewers*

Eric Lombaert, Aurelie Blin, Barbara Porro, Thomas Guillemaud, Julio S Bernal, Gary Chang, Natalia Kirichenko, Thomas W Sappington, Stefan Toepfer, Emeline Deleury (2025) Unraveling genetic load dynamics during biological invasion: insights from two invasive insect species. bioRxiv, ver. 3, peer-reviewed and recommended by Peer Community in Evolutionary Biology. `https://doi.org/10.1101/2024.09.02.610743`

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We live both in a worrying and fascinating time. Worrying because human-induced global change has dramatic consequences on biodiversity around the world. Fascinating because these changes enable us to witness evolutionary processes unfolding on relatively short time scales. One such process is biological invasion. An intriguing evolutionary question is to understand which factor facilitates the success of an invasive species. In particular, serial bottlenecks at the expanding front should reduce the effective population size and decrease genetic diversity. Theoretically, this will increase the fixation of deleterious mutations due to the effect of genetic drift and overall affect the evolutionary potential of the invading species. In the short term, reduced genetic diversity and inbreeding in small populations increases the number of recessive deleterious variants exposed in a homozygous state. This may generate a reduction in mean fitness of the population. However, in the long term and under specific demographic scenarios recessive deleterious alleles may be more efficiently removed by purifying selection. Such purging may explain the success of invasion by reducing inbreeding depression and minimizing loss of fitness. Here, Lombaert et al. estimate the genetic load in two invasive insect species, a predator species, the harlequin ladybird (*Harmonia axyridis*) and a crop pest species, the western corn rootworm (*Diabrotica virgifera virgifera*).

The authors smartly took advantage of a pool-seq transcriptome-based exome capture method to estimate genetic load and assess the purge hypothesis using standard population genetic statistics, such as the ratio of nonsynonymous over synonymous expected heterozygosity, the frequency of derived alleles, and their excess or deficit.

The results revealed different patterns in the two species:

In the western corn rootworm, the authors find a clear signal of reduced genetic diversity in invasive populations. This was associated with a slightly reduced genetic load. However, there was only marginal evidence of purging regarding the most deleterious mutations, and in a single population, with moderately deleterious variants being weakly purged, as theoretically expected.

In the harlequin ladybird, in contrast, the reduction of genetic diversity in invasive populations has been small, a result related to the mild severity of the bottlenecks. In this species, the authors found a tendency toward fixation of the genetic load and no signal of purging.

Such results are intriguing, showing that different species seem to exhibit contrasted fate of genetic load. Differences in the invasion history and ecology of the species may explain these patterns. This is one of the first studies to use a population genomics approach to study the genetic load associated with biological invasion. Future studies based on whole genome data collected at the individual level across multiple species are needed to better understand the dynamics of genetic load during biological invasion and to draw more general conclusions. Advances in forward simulations may also be used to shed light on the evolution of the genetic load at different stages of the invasion process and under different strengths of bottlenecks.

References:

Eric Lombaert, Aurelie Blin, Barbara Porro, Thomas Guillemaud, Julio S Bernal, Gary Chang, Natalia Kirichenko, Thomas W Sappington, Stefan Toepfer, Emeline Deleury (2025) Unraveling genetic load dynamics during biological invasion: insights from two invasive insect species. bioRxiv, ver.3 peer-reviewed and recommended by PCI Evol Biol `https://doi.org/10.1101/2024.09.02.610743`

Reviews

Evaluation round #2

DOI or URL of the preprint: `https://doi.org/10.1101/2024.09.02.610743`
Version of the preprint: 2

Authors' reply, 26 February 2025

Download author's reply

Decision by Quentin Rougemont, posted 20 February 2025, validated 21 February 2025

This revision is a great improvement and I agree with the reviewer that it addresses all of the previous concerns.

I encourage the authors to take into account the two final very small comments about the history of weighting ancestral states as a function of probabilities and the very small as well as rephrasing the L85, as pointed out by Reviewer 1.

I do not anticipate that any further review is necessary and after these modifications I'll be able to recommend the article

Reviewed by **Sylvain Glémin**, 19 February 2025

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.

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]

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).

Reviewed by anonymous reviewer 1, 30 January 2025

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.

Evaluation round #1

DOI or URL of the preprint: `https://doi.org/10.1101/2024.09.02.610743`

Version of the preprint: 1

Authors' reply, 28 January 2025

Download author's reply

Decision by **Quentin Rougemont**, posted 02 October 2024, validated 03 October 2024

Dear authors,

The manuscript entitled "Unraveling genetic load dynamics during biological invasion: insights from two invasive insect species" by Lombaert et al has been evaluated by two referees. They agree that this study is of great interest, both from a theoretical and applied standpoint. However, the two reviewers also raised some concerns that I am also sharing. In particular I suggest the reviewers follow the suggestions of weighting each SNP as a function of the probability of the ancestral state, to reduce uncertainty around polarization, to better assess or present the robustness of the RXY according to the inferred demographic history and to provide results for synonymous SNP. Related to demographic history, more details would be welcome: in the supplementary ABC-RF analyses, the different scenarios do not allow for any gene flow, which is surprising. More importantly, no estimates of the robustness of the RF classifier is provided, nor any estimate of the classification error, whereas the latter is readily available in the ABC-RF package. These details are much needed.

Second, estimates of the levels of inbreeding (e.g. FROH) would be useful, as suggested by reviewer 2. In addition, the reviewer #2 also asks for founder population size estimates, I agree that it would be great if such estimates are available.

Most importantly, reviewer #2 asks to better present the hypothesis/goal being tested (in the abstract/introduction) and whether this can help or not assess the load's role during biological invasion.

Based on reviewer #2 comments extensive work on the distinction between purging by drift versus purging by inbreeding is needed. A careful reading of the manuscript to ensure proper terminology is used would be welcome.

Finally, I was surprised to see a mention of the expansion load concept in the discussion but to see nothing in the introduction: is it relevant to the concept of biological invasion? If yes, how ? how does the expansion load hypothesis compare with (contradicts?) the purging hypothesis ? At least, either provide more details or remove references to this concept.

The statement of a "reduced efficacy of selection" (Line 370), based on the sole observation of HeN/HeS ratio seems a bit hasty. In addition, if the selection efficacy is reduced, shouldn't we expect a stronger load, at least at the early stage of the invasion process, before any purge could take place ?

In summary I strongly suggest the authors to carefully consider all the reviewers' recommendations.

I would be happy to reconsider this manuscript for a recommendation in PCI Evol Biol if the authors can address point by point the reviewer's comments.

Best wishes

Quentin Rougemont

Reviewed by anonymous reviewer 2, 21 September 2024

Review of the manuscript entitled « Unraveling genetic load dynamics during biological invasion: insights from two invasive insect species" by Lombaert et al.

In this article, the authors use a population genomic approach to study the effect of invasion history on the mutation load in two insect species, the western corn rootworm (Diabrotica virgifera virgifera, DVV hereafter and in the manuscript) and the harlequin ladybird (Harmonia axyridis, HA hereafter and in the manuscript). In particular, the aim is to test whether purging of the load has occurred during invasion because of episodes of bottlenecks. To do so, they sampled native and invasive populations of each species plus two/three outgroups to polarize mutations and use exome capture and pool sequencing. They annotate the deleteriousness of non-synonymous SNPs and compare patterns of genetic diversity and the load between native and invasive populations. The two species show contrasted patterns: they found a clear reduction in genetic diversity in DDV but no clear evidence of purging whereas in HA the reduction in diversity is weak and there are signs of increased load.

The manuscript addresses an important question, for both a theoretical point of view in evolutionary biology and a more applied one to understand the dynamics of invasive species. The experimental design is well thought, in particular using two or three outgroups allow polarizing alleles confidently, which is a key part of the analysis. Results are cautiously discussed and not over-interpreted. However, I have a few reserves about the analyses. I don't think it should affect the main patterns but I think some points should be clarified and I also suggest some re-analyses.

Polarization of SNPs

- Using two or three outgroups and the est-SFS tool is a robust approach to polarized SNPs. However, I don't understand why the authors repeated the polarization procedure for each native population separately instead of doing a single polarization. This constrained them to combine the results making some arbitrary choices about the thresholds.
- Even with a single polarization, est-SFS gives a probability of being ancestral. However, the threshold of 0.75 is not very stringent. In addition, using a threshold can sometimes biased the results. One solution would be to test different thresholds to assess the robustness of the results. Another, and I think better, approach would be to weight the SNPs as a function of the probability of ancestral state. For example, a mutation in frequency x with a probability p to be derived should count as p for frequency x and (1 – p) for frequency 1 – x. Or equivalently, the ancestral state could be randomly sampled with probability p / 1 – p.

Rxy analysis

- For DDV, the Rxy results differ among populations (even if only one case is significant). However, the reference population varies. This is based on the history of invasion but what is the robustness of the scenario?
- As a control it could be useful to also give the results for synonymous SNPs

Discussion

- The interpretations of mean allelic frequencies can be partly misleading. The load and inbreeding depression are not linear function of allelic frequencies, so they also depend on the variance (see for example, Bataillon, T., and M. Kirkpatrick. 2000. Inbreeding depression due to mildly deleterious mutations in finite populations: size does matter. Genetics Research 75:75–81). For example, the Hungarian population of DDV shows a signature of purging (Rxy < 1) but on figure 2, it seems that it has more fixed deleterious mutations and fixed mutations generate a load disproportionally larger than those maintained in low frequency (s, selection coefficient, versus u, mutation rate).
- The results suggest that HA may have accumulated instead of purged deleterious mutations, which is at variance with the results of Facon et al. (2011) based on direct fitness measures. However, this can also be explained by the effect mentioned above. Alternatively, very strongly deleterious mutations that can contribute to inbreeding depression can be maintained in too low frequencies to be detected in the samples. For example, assuming a mutation rate of 10^-6, a recessive lethal is expect to segregate in frequency sqrt[u] = 1/1000 in a large panmicitic population, which is almost impossible to capture with a sample size of 80.

Minor comments

- L77-78: This situation often results in a ”mutational meltdown”. This is a theoretical expectation but to my knowledge there is very few empirical evidence of mutational meltdown in natural conditions. Please some empirical examples or rephrase this sentence: for example, “may result” instead of “often results”.
- In the different figures, it would help the reader to more clearly mark the difference between native and invasive populations. There is only the letter N and I in the middle of other letters.

Reviewed by anonymous reviewer 1, 24 September 2024

Dear Editor and authors,

Thank you for the opportunity to review the manuscript entitled 'Unraveling genetic load dynamics during biological invasion: insights from two invasive insect species'. I had great pleasure reading and reviewing the manuscript and provide detailed comments below.

I hope my comments will be helpful and will contribute to the improvement of the manuscript.

Best Regards.

Editorial questions:

Title and abstract

Does the title clearly reflect the content of the article? [X] Yes, [ ] No (please explain), [ ] I don't know

Does the abstract present the main findings of the study? [X] Yes, [ ] No (please explain), [ ] I don't know

Introduction

Are the research questions/hypotheses/predictions clearly presented? [ ] Yes, [X] No (please explain), [ ] I don't know

»> As stated in my comments, there should be a clear distinction between reduction in genetic load via drift or purging (i.e. inbreeding facilitating purifying selection). It is also not clear how the authors actually test for purging. Data on inbreeding should also be presented.

Does the introduction build on relevant research in the field? [] Yes, [X] No (please explain), [ ] I don't know

»> Overall, yes. However, more information on the colonisation history, number of introductions, founder (effective) population sizes should be discussed in the introduction, if available.

Materials and methods

Are the methods and analyses sufficiently detailed to allow replication by other researchers? [X] Yes, [] No (please explain), [ ] I don't know

Are the methods and statistical analyses appropriate and well described? [X] Yes, [ ] No (please explain), [ ] I don't know

Results

In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? [ ] Yes, [ ] No (please explain), [X] I don't know

Are the results described and interpreted correctly? [] Yes, [X] No (please explain), [ ] I don't know

»> As stated above and in my comments, the authors seem to think that any loss of deleterious variation is evidence for purging. However, drift can also induce a loss in deleterious variation. The interpretation of the results should thus be clarified.

Discussion

Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? [ ] Yes, [X] No (please explain), [ ] I don't know

»> shortcomings related to estimates of inbreeding (e.g. FROH) and a reduced set of genes should be discussed and highlight how to test better for evidence for purging of load. The authors should also discuss the advantage of using forward simulations (e.g. Slim) to recapitulate and test the invasion history of those 2 species.

Are the conclusions adequately supported by the results (without overstating the implications of the findings)? [X] Yes, [ ] No (please explain), [ ] I don't know

In this study, Lombaert et al. quantify genetic diversity and load in two invasive insect species using exome data. They find no strong evidence for purging in one species and an excess in deleterious alleles in the other one, highlighting the contrasted dynamics of load during the invasion process.

Overall, I think it is a very interesting study and it is great to read a paper on the dynamics of load during the invasion process. However, while the manuscript is well written and the data analyses are overall sound, there seems to be some degree of confusion regarding what purging actually is. Some clarification is thus needed on what purging is. Furthermore, I think it should be more clear whether the authors test for purging of load and how it can contribute to the invasion success. Reading the abstract, it seems that they are testing this hypothesis, but in the introduction, they stress that it is not what they are testing. So this should be clarified. In light of these clarifications, the discussion of the results should be updated, making clear whether the pattern observed is the result of purging, drift or a combination of both.

Main comments:

First of all, I think that the way the hypothesis to be tested is formulated is not completely correct. The authors refer to the 'so-called purge hypothesis' in the abstract. I would avoid using the 'so-called', especially if no reference to this hypothesis is given. Furthermore, I would avoid referring to 'intriguing' on l. 73 as this hypothesis is not new at all and has been tested before many times, even before the era of genomics.

Also, on l-73-86. The way purging is referred to is a bit unclear. As it reads now, it suggests that purging is a process that can occur immediately at the start of the invasion and it almost reads as if loss of deleterious variation is always caused by purging. The latter is incorrect and it will take several generations for inbreeding to increase and facilitate the exposure of deleterious variation in homozygous state and thus to selection.

See definition from Dussex et al. (2023):
Purging: reduction in genetic load by purifying selection operating against recessive deleterious variants exposed in a homozygous state due to inbreeding in small populations, through population fragmentation or under positive assortative mating.

Similarly, this statement is rather vague and incorrect: 'Conversely, bottlenecks may also purge deleterious alleles, thereby increasing the mean fitness of the introduced individuals.' It is not the bottleneck that will purge load, but natural selection, aided by increased inbreeding.

I would thus reframe this paragraph (and abstract) and instead mention how drift and/or purifying selection + inbreeding (i.e. purging) can contribute to the reduction in genetic load. While the two processes are linked and can produce the same effect, they are not the same.

Related to this point, if one really wants to assess whether purging occurred or not, estimates of inbreeding (FIS) or better based on Runs of Homozygosity (FROH) would be needed. While the latter may not be possible with transcriptome-based exome data, FIS should be estimated here based on synonymous variation. I would also show boxplots with the number of homozygous and heterozygous alleles for each category, after filtering the data for missing sites. This will provide a visually explicit representation of masked and realised load. In addition, it would be to estimate realised and masked load as described in Mathur&DeWoody (2021) - Genetic load has potential in large populations but is realized in small inbred populations.

Secondly, there is not information on the founder population sizes for these two species. Is there information available from previous studies? Do we know if these introductions were serial or a unique event? I think this would be really important to add because it is crucial to understand the dynamics of load.

Minor comments:

l. 62-63. 'The key factors determining the success of invasive species remain largely hypothetical'. Do they really? I would have thought that at least some of those factors would be well known (e.g. absence of natural competitor). Maybe this statement needs to be rephrased or toned down or a reference should be given to support it.

l. 65. Is 'simultaneously' needed here?

l. 78. I may be good to mention that 'mutational meltdown' also refers to an increase in the expression of deleterious variation which may increase the risk of extinction.

l. 94. '...did not experience the inbreeding depression suffered'. Please rephrase such as 'affected by inbreeding depression' or 'showing evidence of inbreeding depression'

l. 96. I would use 'dynamics of load' instead of 'evolution of load'.

l. 99. 'past' instead of 'last'

l. 101. 'applied to' instead of 'expanded into the fields of'

l. 105. 'reduction' instead of 'losses'

l. 107 'High quality genomic resources' instead of 'A good knowledge of the genome'

l. 107-112. I would suggest to maybe rephrase these few sentences along the lines of 'while WGS used to be prohibitively expensive, it has now become more affordable/routine and progress in bioinformatics analyses have also improved greatly', or something along those lines.

l. 112. I would rephrase and say either 'test the hypothesis of purging during the process of invasion' or 'examined the dynamics of load in two invasive species...'

l. 114. 'measuring/estimating load' instead of 'assessing'

l. 115. I am not sure I understand this statement: 'Importantly, the purpose of this study did not include testing the instrumental role of purging in invasion success'. Based on the introduction and the previous statement, I would have thought that assessing the role of purging in the success or history of invasion was the main goal of the study. Surely, if load is purged rapidly, this would facilitate the invasive potential (along with other processes, e.g. absence of natural predator, etc...). So maybe it could be rephrased along the lines of 'assessing the potential contribution of purging to invasion success'. If one species shows evidence or purging and the other not, but the 2 are 'equally' successful, maybe purging is not necessarily needed? This will of course depend on the founder population size, but this may provide clues on the role of purging in the invasion success.

l. 119-121. Maybe state 'the fate of the genetic load during the invasion process' or similar.

l. 128 and elsewhere: 'purging of genetic load' instead of 'genetic load purging'

l. 287. Could you give more detail on what "essential information from the vcf file' refer to?

l. 387 'In all populations studied and for each species'

l. 463. Use 'dynamics' instead of 'fate'

l. 470 maybe add 'and excess in LoF variants, albeit non-significant'? This may apply to l. 476-478 as well.

l. 478 'changes' instead of 'evolution'

l. 486-468. 'This suggests that the demographic and selective constraints in this population were effective at purging highly deleterious mutations but perhaps not moderately deleterious ones.' This goes back to main comment. This should be clarified and the distinction between drift and purging (selection) should be clear. If you cannot show that purging actually facilitated the reduction of load, you can state that this reduction was facilitated by a combination of drift and purifying selection. This is also why some measure of inbreeding is important to add.

L. 490. Replace 'significant' with 'severe'

l. 494. Again, it is not clear whether purging or drift facilitated this loss of deleterious variation.

l. 504-507. This would give more support for drift leading to fixation of deleterious alleles and should be mentioned here.

l. 535. 'our study is one of the first in the context of biological invasions' . Could the authors cite a few of those studies? What have they shown? Have they assessed the role of purging on the invasion success?