Review of manuscript: *How do plant RNA viruses overcome the negative effect of Muller's ratchet despite strong transmission bottlenecks?*

The authors here have done extensive experiments to evaluate the effects of bottlenecks on the fitness of Tobacco Etch Virus in its natural host, Nicotiana tobacum. The hypothesize that Muller's ratchet, the irreversible accumulation of deleterious mutations, is responsible for the fitness loss they observe when narrow bottlenecks are imposed between generations of the virus. They also include the use of a model to explore population dynamics within single TEV-induced lesions found on the host plant. The authors point out that while similar experiments have been done in other viral systems, this is the first time it is being done in plant viruses which are often neglected. I agree that these experiments present a novel and important contribution to the field.

I think that if the authors address some of my major concerns, this manuscript will be ready for publication. The review is presented in the context of some prompting questions provided by PCI.

Concerns needing <u>major</u> revision

Is the experimental plan consistent with the questions?

> They perform infectivity analysis on N. tabacum. Their "weak bottleneck" lineages were the only ones passaged on that plant, making it difficult to tell whether the effects they observe between lineages are due to differences in selection scheme (environment) or demography (larger population sizes between passages). I believe the scope of the question getting at this concern was outlined in lines 90 to 93. However, I think additional text could be added stating the importance of using C. quinoa as one of the additional experimental hosts. I understand they cite work describing the decrease in fitness of TEV in C. quinoa, but I am curious if there is work describing whether this occurs in the N. tabacum system when there are strict bottlenecks between passages. If not, why not?
>
> Some literature that comes to mind when thinking about mutations in fluctuating environments:
>> https://doi.org/10.1073/pnas.1505406112
>> https://doi.org/10.1111/j.1558-5646.2012.01680.x
>> doi:10.1111/j.1558-5646.2010.01051.x
>
> The authors briefly address the use of multiple hosts in lines 391 and 392, but I think adding similar text in the introduction would help readers with similar concerns know that this is a feature, not a flaw, of the experiment.

Are the statistical analyses appropriate?

> I really appreciated the analysis of lesions against the infectivity of N. tabacum. This was a helpful point to make, and the authors may consider moving this figure from the supplement to the main text if they desire. I think it could fit nicely in Figure 2 or as its own figure but discussed earlier on in the results.
>
> The statistical analyses throughout the paper seem reasonable given their experimental setup with the exception of two sections:

Review of manuscript: *How do plant RNA viruses overcome the negative effect of Muller's ratchet despite strong transmission bottlenecks?*

I)   Genetic Variance

The authors discuss "significant" changes in variance in the section beginning on line 233, Variance intra- and inter-lineages. However, this was not accompanied by any statistical test or approximate justification. Additionally, the use of these variances was omitted in the Methods section. Finally, I am having a difficult time interpreting the Figure 3 plots. Their description of Figure 3B seems to ignore a particular datapoint where the intermediate population appears to have a decrease in variance during one of the passages but the authors claim "the intermediate and large Ne lineages did not display any significant changes in intra-lineage variation throughout the passages."

II)   Simulation Model

I enjoy the inclusion of the simulations in the manuscript. I think the setup makes intuitive sense as someone familiar with these types of models. They appear to adequately search parameter space for reasonable parameters. However, to make their quantitative methods more rigorous, I would have liked to see the authors fit their model to their data. They claim that the model trends (Figure 5) match the data well (Figures 2 and 3). However, the authors could make a more convincing claim if they fit their model to data so the actual values of the model and data match – they don't match in 5A and 2B. My observation that they don't match by eye might be evidence that a better-fit parameterization is available in parameter space.

I suggest the authors look into particle MCMC as a potential method for fitting this stochastic model to their data. With this method they will be able to estimate model parameters from their data, potentially showing where the small and intermediate Ne experiments disagree. A good place to learn about this method is this Endo et al paper, https://doi.org/10.1016/j.epidem.2019.100363. A couple of examples of its use in the literature can be found in Zhu et al, https://doi.org/10.3390/v13071216, and Rasmussen et al, doi:10.1371/journal.pcbi.1002136.

Concerns needing <u>minor</u> revision

<u>Line 38</u>: The authors have several citations for estimates of viral mutation rates. However, I would advocate for the inclusion of a slightly more contemporary citation among them since methods for estimating mutation rates have been updated. Perhaps 10.1128/JVI.01031-17 or 10.1007/s00018-016-2299-6 could be a good fit.

<u>On lines 83 to 85</u>: The authors claim TEV does not migrate within the plant. However, the presence of lesions suggests local spread, and their aphid hosts may allow for migration or multiple transmission events in nature. Perhaps a point to address in the discussion.

Figure 6 in the discussion is labeled Figure 5.

Be aware that the hyperlink on line 162 of the PDF pointing to the simulation model on Zenodo goes to a broken page. The hyperlink text is correct and works on the full text version on bioRxiv.

Review of manuscript: *How do plant RNA viruses overcome the negative effect of Muller's ratchet despite strong transmission bottlenecks?*

Is the manuscript well written?

> While there is incorrect grammar in some sentences of the article and README file, the clarity of the message is not hindered by these small errors. Example in the README file: "A few number of particle initiate a lesion" could be changed to "A small number of particles initiate a lesion" or "A few particles initiate a lesion." It seems most errors are made when using the plural and the future tense. To be ready for publication, I suggest passing the next version of the manuscript through software like Grammarly or ChatGPT to help address some of these small grammatical issues.

Do the tables and figures have suitable captions such that they can be understood without having to read the main text?

> I would favor longer figure captions to address this question. It may help more readers understand the importance of each figure, which is difficult to do in particular for Figure 3.