



Temperature effects on virulence evolution of wMelPop *Wolbachia* in *Drosophila melanogaster*

[Ellen Decaestecker](#) based on reviews by Shira Houwenhuyse and 3 anonymous reviewers

A recommendation of:

David Monnin, Natacha Kremer, Caroline Michaud, Manon Villa, H  l  ne Henri, Emmanuel Desouhant, Fabrice Vavre. **Experimental evolution of virulence and associated traits in a *Drosophila melanogaster* – *Wolbachia* symbiosis (2020)**, *bioRxiv*, 2020.04.26.062265, ver. 4 peer-reviewed and recommended by Peer Community in Evolutionary Biology. [10.1101/2020.04.26.062265](https://doi.org/10.1101/2020.04.26.062265)

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Monnin et al. [1] here studied how *Drosophila* populations are affected when exposed to a high virulent endosymbiotic wMelPop *Wolbachia* strain and why virulent vertically transmitting endosymbionts persist in nature. This virulent wMelPop strain has been described to be a blocker of dengue and other arboviral infections in arthropod vector species, such as *Aedes aegypti*. Whereas it can thus function as a mutualistic symbiont, it here acts as an antagonist along the mutualism-antagonism continuum symbionts operate. The wMelPop strain is not a natural occurring strain in *Drosophila melanogaster* and thus the start of this experiment can be seen as a novel host-pathogen association. Through experimental evolution of 17 generations, the authors studied how high temperature affects wMelPop *Wolbachia* virulence and *Drosophila melanogaster* survival. The authors used *Drosophila* strains that were selected for late reproduction, given that this should favor evolution to a lower virulence. Assumptions for this hypothesis are not given in the manuscript here, but it can indeed be assumed that energy that is assimilated to symbiont tolerance instead of reproduction may lead to reduced virulence evolution. This has equally been suggested by Reyserhove et al. [2] in a dynamics energy budget model tailored to *Daphnia magna* virulence evolution upon a viral infection causing White fat Cell disease, reconstructing changing environments through time.

Contrary to their expectations for vertically transmitting symbionts, the authors did not find a reduction in wMelPop *Wolbachia* virulence during the course of the experimental evolution experiment under high temperature. Important is what this learns for virulence evolution, also for currently horizontal transmitting disease epidemics (such as COVID-19). It mainly reflects that evolution of virulence for new host-pathogen associations is

difficult to predict and that it may take multiple generations before optimal levels of virulence are reached [3,4]. These optimal levels of virulence will depend on trade-offs with other life history traits of the symbiont, but also on host demography, host heterogeneity, amongst others [5,6]. Multiple microbial interactions may affect the outcome of virulence evolution [7]. Given that no germ-free individuals were used, it can be expected that other components of the *Drosophila* microbiome may have played a role in the virulence evolution. In most cases, microbiota have been described as defensive or protective for virulent symbionts [8], but they may also have stimulated the high levels of virulence. Especially, given that upon higher temperatures, *Wolbachia* growth may have been increased, host metabolic demands increased [9], host immune responses affected and microbial communities changed [10]. This may have resulted in increased competitive interactions to retrieve host resources, sustaining high virulence levels of the symbiont.

A nice asset of this study is that the phenotypic results obtained in the experimental evolution set-up were linked with wMelPop density measurement and octomom copy number quantifications. Octomom is a specific 8-n genes region of the *Wolbachia* genome responsible for wMelPop virulence, so there is a link between the phenotypic and molecular functions of the involved symbiont. The authors found that density, octomom copy number and virulence were correlated to each other. An important note the authors address in their discussion is that, to exclude the possibility that octomom copy number has an effect on density, and density on virulence, the effect of these variables should be assessed independently of temperature and age. The obtained results are a valuable contribution to the ongoing debate on the relationship between wMelPop octomom copy number, density and virulence.

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Revision round #1

2020-06-05

Dear Dr. Monnin and colleagues,

I obtained now four revisions of your manuscript. While all reviewers indicate that your study is valuable with respect to the role of virulence evolution of symbionts in changing environments, they also have some comments, some of which are quite substantial. I send you the reviewer report from reviewer 1 below and the other 3 reviewers in attach. Could you address the issues raised by the reviewers and then I suggest you send a revised manuscript with a rebuttal letter how you addressed the issues raised by the reviewers. If you wish no longer to resubmit to PCI I understand this, but then I hope the revisions are helpful for your further submissions.

Kind regards, Ellen Decaestecker

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Reviewed by [Shira Houwenhuys](#), 2020-05-06 09:56

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Reviewed by anonymous reviewer, 2020-05-26 16:48

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Reviewed by anonymous reviewer, 2020-06-04 19:38

This manuscript addresses the hypothesis that vertically transmitted symbionts should evolve low virulence as detrimental effects of the symbiont on the host would decrease fitness of the host, and consequently also the endosymbiont. The wMelPop Wolbachia strain that infects *Drosophila* flies indeed show such an expected low virulence, except at higher temperatures. It has been proposed that this is likely non-adaptive as flies are generally not found at such high temperatures. The authors use this setting to conduct an evolution experiment to test if selection for late reproduction results in the evolution of lower virulence. Interestingly, the virulence of wMelPop can be reduced by supplementing Paraquat to the medium.

Although this is an interesting idea and setup, it is not always clear how adequate the experiment is to test this idea. The writing and organization of this manuscript is generally highly unclear and very difficult to follow.

1/ Virulence of Wolbachia at high temperatures is reflected by a strong decrease in host longevity. However, the selection experiment was conducted by selecting for late reproduction, and even this selection procedure is doubtful. Why did the authors not simply select for flies with high survival rate? The survival experiment shows that flies indeed start to show (a very low level) of mortality at the time eggs were selected (8 days after emergence). However, how do the authors know that the eggs selected at that time originate from flies with increased longevity? If this is not the case, it should be no surprise that no effects of treatment are observed or if even any type of selection was performed.
2/ L231-237: This is very unclear. The first sentence describes that density and mean octomom copy number did not increase during experimental evolution, but the next sentence describes that it increased? If this latter refers to an increase between the treatments, this should be clearly stated, as well as for which of the

two treatments the increase was observed. I also would expect that the interaction generation x treatment should be the factor demonstrating an effect of selection, but this is not interpreted as such. On L279 the authors state that 'the increase of time in density and copy number....', which refers to a generation x treatment interaction, but this was not significant. I'm also surprised to note that the significance levels are so high, as the graphs (Fig. 3) hardly show a clear effect because of the very large variance between the lines.

3/ More minor comments:

- a. The results and discussion are completely mixed up. For example, the authors interpret the results of the survival rate comparison directly as virulence comparison. It is much more accurate to describe the results of survival as such, and afterwards discuss how this can be interpreted as virulence.
- b. b. The phrasing of the co-evolution between symbionts and hosts is often scientifically incorrect and teleological. E.g. l33-34: endosymbionts exploit their host to maximize their transmission. This phrasing suggests that they evolved this 'on purpose', which is not the case in evolution. Mean trait values evolve as a consequence of fitness difference, but not for any purpose.
- c. L206-207: Clearly state for which response variable a significant effect was found. E.g. "...did not modify the evolution of virulence" , but as different response variables are used as proxy for virulence, readers cannot infer about which response variable the authors are writing.

Reviewed by anonymous reviewer, 2020-05-26 15:38

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Author's reply:

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