

Review: Experimental evolution of virulence and associated traits in a *Drosophila melanogaster* – *Wolbachia* symbiosis.

General comments

David Monnin and colleagues investigated virulence evolution of the symbiotic *Wolbachia* strain wMelPop in *Drosophila* under high temperature (at which the strain is assumed to be virulent) and in the presence or absence of paraquat. Their hypotheses were well-supported by theoretical expectations regarding the evolution of reduced virulence of vertically transmitted symbionts. The introduction is well-written with clear predictions regarding the experiment. These predictions were, however, not confirmed by their experiment and their main hypothesis - the evolution of reduced virulence - was not confirmed. The discussion provides the alternative explanation that intra-host selection might be important here, and I agree that it might possibly play a role here.

I do have some major concerns about how the evolution of virulence was measured. First, from the manuscript it is not clear which survival parameter you compare between G3 and G9. Time until half of the flies died? mean survival time? I think survival analysis would be the best option here. I also wonder if using only fly survival of wMelPop infected flies is a good proxy for virulence? I think that virulence is best measured as the reduction in survival compared to an uninfected control. This way you also correct in some way for measuring survival on different days (is it possible that e.g. differences in food quality between days can be a confounding factor?). Ideally you would even isolate different *Wolbachia* lineages and re-infect flies to test everything at once (but maybe this is technically not possible?). My feeling is that possibly some patterns were not found because of the way virulence was measured.

I also have a general suggestion on providing more information on the population dynamics of wMelPop. I think some information is crucial here to understand how selection might act on these bacteria. Is there a population bottleneck at reproduction/how many bacterial cells are transmitted to the egg?, What is the number of

generations within a host? Especially in the light of intra-host selection this might be very important.

Specific comments

Line 60: do you have more functional information on the genes contained in 'octomom'?

Line 64: Here it is indicated that this *Wolbachia* strain is not known to occur in nature. Could you then give a little more explanation where it comes from?

Line 91: I think it is better to mention the experimental temperature in the next section.

Line 103: Why do you use unsupplemented medium for the paraquat lines in the egg-laying phase of the experiment?

Line 104: Is it known if wMelPop affects reproduction of the flies? If so, this might actually be a stronger selective force for reduced virulence than longevity.

Line 168: Maybe best to quickly describe here why you do this (it is only clear when reading the last section of the discussion).

Line 202: In the absence on mortality from non-infected flies it is very difficult to say that this mortality is induced by the *Wolbachia*.

Line 213: "virulence possibly decreased subsequently" is a bit weird here, as this is shown not to be the case in line 217.

Line 215: Can you statistically test differences in extinction rates for different conditions?