Limits to modeling the (thermal) limits of *Wolbachia*

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Abstract

Wolbachia release programs with the *w*Mel strain are suppressing the incidence of dengue following releases in many countries. Vasquez et al[1] use models to predict the impact of increasing temperatures and heatwaves on the replacement of wild mosquito populations with *w*Mel carriers that are poor dengue vectors. They claim that *w*Mel replacement is resilient to pre-2060 climate change including anomalies, although sustained heatwaves may still threaten release programs. Here, we suggest that their assumptions which are extrapolated from laboratory experiments may be unrealistic and overly simplistic because they only focus on temperature. Furthermore, there are already environmental constraints on *Wolbachia* release programs under existing climates. We urge caution when making predictions about the likely success of specific dengue control programs and emphasize that models must consider the full extent of environmental effects on *Wolbachia* and its mosquito host, with experiments and modeling parameters directly linked to current and future microenvironmental conditions.

Main text

The release of *w*Mel and *w*AlbB *Wolbachia*-carrying *Aedes aegypti* mosquitoes in the tropics is providing an effective biological control against dengue. Both infections block the transmission of arboviruses by mosquitoes and, providing the infection can be maintained in populations, there can be massive impacts on dengue incidence in human populations likely to persist for many years[2]. But with the rollout of *w*Mel in a range of countries and *w*AlbB at different sites, complexities and challenges are emerging due to variation in local environments. Research from our group[3] and others[4] has described the sensitivity of *w*Mel to high temperatures, which we expected to impact *Wolbachia* population replacement in some environments. Observations from semi-field experiments and recent field releases in Nha Trang, Vietnam[5] are consistent with temperature being an important factor influencing *Wolbachia* replacement. But there are other important environmental factors, including context-dependent fitness costs of *Wolbachia* on egg quiescence[6] and effects of host genotype[7]. The complex nature of *Wolbachia* replacement is best demonstrated by the fact *Wolbachia* persistence can differ dramatically across release zones within the same city, despite similarity in ambient climate[5, 8].

Limitations of parameters based on laboratory data

The models in Vasquez et al[1] are primarily based on a thermal threshold for *w*Mel of 35°C, with daily average (or 7 day rolling average) temperatures above this point assumed to cause maternal transmission and cytoplasmic incompatibility (embryo sterility from crosses between infected males and uninfected females) to fall to zero. Both maternal transmission and cytoplasmic incompatibility are key features of *Wolbachia* essential for population replacement as first shown in field *Drosophila* populations[9]. The 35°C threshold is based on our laboratory experiments[10] where *w*Mel is lost when eggs are exposed to a diurnal temperature cycle of 30-40°C for one week. However, a thermal threshold of 35°C in nature is unrealistic for several reasons. (1) Cycling temperatures around a mean are not equivalent to constant temperatures at that mean, particularly when heat stress typically accumulates in an organism[11]. (2) Other life stages are more vulnerable to the effects of heat on *w*Mel so using data from eggs is likely to overestimate the threshold. (3) Effects of high temperatures can accumulate when the exposure time is increased, and effects can persist across generations. We have shown previously that *w*Mel density can decrease even at moderate temperatures (cycling from 26-36°C) when larvae have an extended developmental period[12]. (4) We did not measure either maternal transmission or cytoplasmic incompatibility in the experiment– these are both affected below thermal limits where *Wolbachia* is lost in the mosquito. While the authors use a presence-absence threshold for *Wolbachia*, the reality is that both cytoplasmic incompatibility and maternal transmission are on a continuum, and the partial loss of *Wolbachia* can have complex effects including partial self-incompatibility[10].

For these reasons, we suspect that the authors have overestimated the temperature threshold of *w*Mel. Moreover, by simplifying the temperature effects to a binary threshold, the models ignore the complex effects of partial loss of *w*Mel on population dynamics. The authors acknowledge the uncertainties in the temperature profile of *w*Mel and therefore perform a sensitivity analysis where they set the threshold at 33°C or 31.5°C, but conclusions remain largely based on the 35°C threshold. Before making predictions about future suitability of thermal conditions for invasion, an understanding needs to be developed about the suitability of *w*Mel (and other strains) under current conditions. Predictions should be validated by tracking *w*Mel replacement across generations under a variety of thermal regimes and in areas with different larval habitats that generate enormous variability in the thermal conditions experienced by early developmental stages of the mosquito[13].

Environmental factors beyond temperature

Vasquez et al[1] assume a fixed 10% fitness cost for *w*Mel carriers relative to wild-type mosquitoes in the main text and do not consider other variables beyond temperature. In the supplementary information, the authors also consider a fixed cost of 20%. However, this is likely to be a conservative estimate, with data from field releases in Cairns estimating costs closer to 30%[14]. In Rio de Janeiro, Brazil where *w*Mel has reached only moderate prevalence in some release sites despite supplementary releases, fitness costs are likely to be even higher and influenced by variables like pesticide resistance in the mosquito genetic background[7]. This will increase the critical threshold for invasion beyond the 20-30% cited by Vasquez et al, which also increases the risk of *w*Mel dropping out due to heatwaves. Fitness costs of *w*Mel are also strongly environmentally dependent. For example, costs of *w*Mel to quiescent egg viability can be substantial[6], providing a massive advantage to wild type mosquitoes in situations where rainfall is infrequent. Models that do not consider these complexities may fail to produce reliable estimates of future climate responses, whereas mechanistic models have been built that allow for the impact of climate change on quiescent egg viability[15].

Model validation

Vasquez et al[1] validate their model with a comparison to two release sites, one where *Wolbachia* has persisted at a largely stable and high frequency for the last decade, and another where there are strong seasonal fluctuations. In Nha Trang, Vietnam, *w*Mel was predicted to drop out only under the assumption of extended heatwaves and their lowest biological threshold for *w*Mel of 31.5°C. However, in release programs, *w*Mel showed strong seasonal changes in frequency and dropped out entirely in two local areas[5]. This indicates that their model overestimates the thermal stability of *w*Mel, and/or ignores other important environmental factors including temperatures in critical larval habitats which may exceed ambient temperatures.

Concluding comments

The models developed by Vasquez et al[1] are a useful tool but their assumptions are too simplistic to provide accurate predictions. They conclude that their models could be extrapolated to other release sites, but they appear to be inconsistent with data from field releases. The authors acknowledge many caveats, particularly around the uncertainty of their assumptions, which means that caution is required in interpreting their findings. Models probing the environmental constraints of climate change on *Aedes* mosquitoes have already been developed[15] and could also be used to guide decisions about where to release *Wolbachia* strains and to provide estimates of their long-term effectiveness. Estimates of *Wolbachia* thermal limits should be based on experiments using conditions that are ecologically relevant, considering the entire mosquito life cycle across multiple generations. As evidenced by the failed establishment of *w*Mel in some release sites[5], there are already limits on where *w*Mel and likely other strains can be used for replacement, regardless of future climate change.

Author contribution statement

PAR wrote the first draft of the manuscript. AAH and PAR revised the manuscript.

Competing interests statement

The authors declare no competing interests.

References

1. Vásquez, V.N., Kueppers, L.M., Rašić, G. & Marshall, J.M. wMel replacement of dengue-competent mosquitoes is robust to near-term change. *Nature Climate Change* **13**, 848-855 (2023).
2. Utarini, A. et al. Efficacy of *Wolbachia*-infected mosquito deployments for the control of dengue. *N Engl J Med* **384**, 2177-2186 (2021).
3. Ross, P.A. et al. *Wolbachia* infections in *Aedes aegypti* differ markedly in their response to cyclical heat stress. *PLoS pathogens* **13**, e1006006 (2017).
4. Ulrich, J.N., Beier, J.C., Devine, G.J. & Hugo, L.E. Heat sensitivity of *w*Mel *Wolbachia* during *Aedes aegypti* development. *PLoS neglected tropical diseases* **10**, e0004873 (2016).
5. Hien, N.T. et al. Environmental factors influence the local establishment of *Wolbachia* in *Aedes aegypti* mosquitoes in two small communities in central Vietnam. *Gates Open Res* **5**, 147 (2021).
6. Allman, M.J. et al. *Wolbachia*'s deleterious Impact on *Aedes aegypti* egg development: The potential role of nutritional parasitism. *Insects* **11** (2020).
7. Garcia, G.d.A. et al. Matching the genetics of released and local *Aedes aegypti* populations is critical to assure *Wolbachia* invasion. *PLoS neglected tropical diseases* **13**, e0007023 (2019).
8. Pinto, S.B. et al. Effectiveness of *Wolbachia*-infected mosquito deployments in reducing the incidence of dengue and other *Aedes*-borne diseases in Niterói, Brazil: A quasi-experimental study. *PLoS neglected tropical diseases* **15**, e0009556 (2021).
9. Turelli, M. & Hoffmann, A.A. Rapid spread of an inherited incompatibility factor in California Drosophila. *Nature* **353**, 440-442 (1991).
10. Ross, P.A., Ritchie, S.A., Axford, J.K. & Hoffmann, A.A. Loss of cytoplasmic incompatibility in *Wolbachia*-infected *Aedes aegypti* under field conditions. *PLoS neglected tropical diseases* **13**, e0007357 (2019).
11. Jørgensen, L.B., Malte, H., Ørsted, M., Klahn, N.A. & Overgaard, J. A unifying model to estimate thermal tolerance limits in ectotherms across static, dynamic and fluctuating exposures to thermal stress. *Scientific reports* **11**, 12840 (2021).
12. Ross, P.A. & Hoffmann, A.A. Continued susceptibility of the *w*Mel *Wolbachia* infection in *Aedes aegypti* to heat stress following field deployment and selection. *Insects* **9** (2018).
13. Richardson, K.M., Hoffmann, A.A., Johnson, P., Ritchie, S.R. & Kearney, M.R. A replicated comparison of breeding-container suitability for the dengue vector *Aedes aegypti* in tropical and temperate Australia. *Austral Ecol* **38**, 219-229 (2013).
14. Hoffmann, A.A. et al. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* **476**, 454-457 (2011).
15. Kearney, M., Porter, W.P., Williams, C., Ritchie, S. & Hoffmann, A.A. Integrating biophysical models and evolutionary theory to predict climatic impacts on species’ ranges: the dengue mosquito *Aedes aegypti* in Australia. *Funct Ecol* **23**, 528-538 (2009).