We recently reanalysed previously published datasets (Souto-Maior et al. and Ferguson et al.). Using mathematical and statistical models based on these data, we showed that Wolbachia strain wMel increases mean and variance of Aedes aegypti susceptibility to infection by dengue viruses. Ant et al. claim that concerns with the data and broader analysis make our conclusions misleading. We herein respond to their comments by demonstrating the robustness of our results to different treatments of the data, and expand our arguments for replacing currently adopted methods by those introduced in our paper.

Ant et al. describe concerns with one of the datasets used in our analysis. They highlight one of the studies and propose a threshold for false positives based on the mean cycle threshold (Ct) value of Aedes aegypti susceptibility to infection by dengue viruses. We next address a comment by Ant et al. that our results contradict other studies. There is no contradiction in the sense that our methods are more general and coincide with those employed by others, when specific assumptions are made. To verify this, suppose that an individual is challenged with d infectious units (pathogen dose) and that each unit has a
probability $p$ of causing infection (host susceptibility). Assuming that the number of units effectively causing infection is Poisson distributed with mean $pd$, the probability of infection is $1-e^{-pd}$. Plotting this probability over a range of doses results in the dose–response curves represented in blue in Fig. 2. In a host population with homogeneous susceptibility, $p$ can be estimated by challenging individuals with doses distributed over a suitable range and fitting the model to the resulting data. We can also relax the condition that the host population is homogeneous and assume that $p$ is distributed according to some model\cite{13}, in which case model fitting will give the distribution of host susceptibilities. Applying this procedure to published data, we concluded that A. aegypti mosquitoes in Brazil\cite{1} performed as homogeneously susceptible to dengue virus infection when challenged by intrathoracic injection with samples isolated from a local patient, whereas another set, with mosquitoes collected in Australia and infective blood drawn from patients in Vietnam\cite{2}, supported gamma-distributed susceptibility under more natural blood-feeding challenges. Ant et al.\cite{4} criticise the inclusion of an intrathoracic injection dataset in our study on the grounds that it represents a substantial deviation from the natural infection route. In performing a comparative analysis between this and a blood-feeding dataset, however, we may be uncovering how much Wolbachia shifts the dose–response curve along the dose axis (in log scale)\cite{9}, carries an implicit assumption that the symbiont affects all individuals equally (Fig. 2a). King et al.\cite{3} showed that neither of these two common approximations (heterogeneous all-or-nothing or homogeneous susceptibility of Wolbachia carriers) was supported by data. Wolbachia does not only contract or stretch dose–response curves along one axis or the other, but changes their shape, suggesting alternative distributions of susceptibility and therefore requiring more sophisticated analyses. This is illustrated in the bottom panels (Fig. 2d, e), which display point estimates of the distributions estimated in King et al.\cite{3} from dengue challenges in Brazil (Fig. 2d) and Vietnam (Fig. 2e). In these cases, Wolbachia was found to increase infection probability at low viral challenge doses (signalling increased mean susceptibility), while leading to shallower
dose–response curves (increased variance) to meet reduction in infected proportions at high doses.

On the issue of uncertainty, Ant et al.4 also comment on the low numbers of mosquitoes positive for dengue virus at low challenge doses, which may result in low statistical power to support dose–response analyses. One of the benefits of fitting a model across a range of doses is precisely to increment statistical power by informing parameter estimation on multiple data points and extrapolating as appropriate13. Numbers infected are inevitably smaller at low challenges unless the experimental groups are incremented to compensate for the lower infection probabilities, but such optimal design strategy will not be adopted unless researchers are aware of its significance. It is entirely possible that large replication efforts might change the best estimates for the susceptibility distributions, and the analysis presented in King et al.3 is not dependent on a particular set of parameter values, or mean and variance effect of Wolbachia. Meanwhile, despite being based on low numbers and small effect sizes, the consistency of higher infected proportions among Wolbachia carriers at low challenge doses is noticeable. Ant et al.4 finalise their inspection into the Souto-Maior et al.1 dataset by noting a slight discrepancy between the raw data and the proportions infected shown in Fig. 1a of King et al.3. This is due to the exclusion of the earliest time point from the analysis (3 days post infection), both for

Fig. 2 Distributions of susceptibility factors and their impact on dose–response curves. Blue dose–response curves represent infection probabilities in insects without Wolbachia, while green represent Wolbachia carriers. Insets depict distributions of susceptibility in noncarriers (blue) and carriers (green) normalised such that noncarriers have mean susceptibility one. Solid black lines represent the mean susceptibility of Wolbachia carriers, while dashed black curves mimic common procedures based on simple arithmetic ratios of the proportions infected dose-by-dose. Top panels (a–c) assume distributions of susceptibility factors with the same mean, less than one (0.2), and different variances (a, 0 (homogeneous); b, 0.0533; c, 0.160 (all-or-nothing)). Bottom panels (d, e) use the susceptibility distributions estimated in King et al.1 (d, mean 1 and variance 0 for noncarriers, and mean 6.92 and variance 143 for carriers; e, mean 1 and variance 2.78 for noncarriers, and mean 1.49 and variance 10.9 for carriers). The threshold separating increased from reduced mean susceptibility is marked by dotted black lines.
reducing uncertainty and for uniformity with Ferguson et al.\textsuperscript{2}. We have inadvertently bypassed this detail in the “Methods” of our original paper.\textsuperscript{3} Ant et al.\textsuperscript{4} present similar arguments concerning our analysis of the Ferguson et al.\textsuperscript{2} dataset, defending dose-by-dose schemes and neglecting low doses, based on the rationale that our paper refutes.

One final point where we agree with Ant et al.\textsuperscript{3} is that “the need for consideration of virus in the saliva is also paramount”, which is why we used a dataset with such an approach\textsuperscript{2} to calibrate our model. This assessment of infectivity was conducted by straightforward procedures requiring only brief description which does not, however, diminish its importance. Integrating estimated Wolbachia effects on both susceptibility and infectivity, our transmission models predict reductions in dengue incidence in Vietnam, while in Brazil the analysis is less conclusive (King et al.\textsuperscript{3}, Fig. 3).

In summary, we recognise that the original paper, especially the Abstract, did not adequately specify that an increase in mean susceptibility to dengue infection due to Wolbachia does not imply higher expected infection probability except at low viral challenge doses. We also did not place necessary caveats on the results noting the limitations of the prior datasets underlying our model (e.g., the existence of a small number of false positives). Nonetheless, we have shown here that our results are robust to a different threshold of data inclusion removing false positives, and that our model based on dose–response relationships reduces to more conventional approaches, when specific assumptions are made.

**Data availability**

All data supporting the findings of this study are available from the original publications Souto-Maior et al.\textsuperscript{1} and Ferguson et al.\textsuperscript{2}.

**Code availability**

Code used in this study is the same as in the original publication King et al.\textsuperscript{3} and can be obtained by contacting the authors.

Received: 17 February 2020; Accepted: 29 October 2020;
Published online: 30 November 2020

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**Acknowledgements**

This work was funded by the Portuguese FCT (IF/01346/2014), Brazilian CAPES (0775/2014) and AXA Research Fund.

**Author contributions**

M.G.M.G., C.S.-M., J.G.K., L.M.S. and R.M.-F. were responsible for the writing of the response. The authors declare no competing interests.