Variation in *Wolbachia* effects on *Aedes* mosquitoes as a determinant of invasiveness and vectorial capacity

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*Wolbachia* has been introduced into *Aedes aegypti* mosquitoes to control the spread of arboviruses, such as dengue, chikungunya and Zika. Studies showed that certain *Wolbachia* strains (such as *w*Mel) reduce replication of dengue viruses in the laboratory, prompting the release of mosquitoes carrying the bacterium into the field, where vectorial capacity can be realistically assessed in relation to native non-carriers. Here we apply a new analysis to two published datasets, and show that *w*Mel increases the mean and the variance in *Ae. aegypti* susceptibility to dengue infection when introgressed into Brazil and Vietnam genetic backgrounds. In the absence of other processes, higher mean susceptibility should lead to enhanced viral transmission. The increase in variance, however, widens the basis for selection imposed by unexplored natural forces, retaining the potential for reducing transmission overall.
Aedes mosquitoes are competent vectors for several viral diseases of humans. Wolbachia is a symbiotic bacterium of arthropods, shown to manipulate the reproduction of its hosts to facilitate invasion of carriers, and to modify host responses to viral infections, notably in Ae. aegypti, indicating a potential role in the control of viral diseases.

Wolbachia is commonly transmitted vertically from mother to offspring. Horizontal transmission between host populations or species is rare, but when successful the spread of the symbiont may be facilitated by multiple mechanisms. First, there are specific forms of reproductive manipulation, such as cytoplasmic incompatibility between sperm of male carriers and eggs of female non-carriers, which result in inviable crosses, suppressing non-carrier populations and, consequently, conferring competitive advantage to Wolbachia carriers. Second, Wolbachia has been shown to confer a degree of protection against viral pathogens, which facilitates its establishment in host populations when such pathogens are present. Third, substantial interindividual variation in this effect has been found, which further increases Wolbachia invasiveness through a process of cohort selection. Cohort selection (also known as survival bias) occurs whenever there is variation and selection, irrespective of whether the trait under study is heritable. In the case of susceptibility to infection (i.e., probability of infection per unit of pathogen challenge), individuals with higher susceptibility are more likely to be affected and therefore tend to be removed from the population at risk sooner than those with lower susceptibility, leading to a decrease in mean susceptibility among survivors as a cohort ages. Thus, by merely increasing variation in host susceptibility to pathogens, Wolbachia increases the resilience of its carrier populations and facilitates its own invasion.

Due to the second and third mechanisms above, Wolbachia invasion implies the replacement of a host population by another whose susceptibility to the pathogen under study is lower. These findings support the notion that Wolbachia carriage by insect vectors of human pathogens, such as mosquitoes, might be manipulated to reduce disease transmission.

The mosquito Ae. aegypti, primary vector of dengue, is not a natural host of Wolbachia. Transfection of various strains has been performed in the laboratory and shown to reduce vector competence, on average, when mosquitoes were challenged with high viral doses. This has led to field releases of wMel-transinfected Ae. aegypti in 10 countries—Australia, Brazil, Colombia, Indonesia, Sri Lanka, India, Vietnam, Kiribati, Fiji and Vanuatu—to evaluate its effectiveness in reducing dengue and other mosquito-borne diseases in human populations.

Here we explore the effects of Wolbachia on the susceptibility distributions of two Ae. aegypti populations—Rio de Janeiro, Brazil, and Ho Chi Minh City, Vietnam—to dengue viruses. The wide variability in exposure doses naturally experienced by mosquitoes prompted the adoption of dose-response experimental design for the inference of distributions of Wolbachia effects, which were then inserted in high-dimensional mathematical models to investigate the conditions for Wolbachia invasion and its impact on dengue transmission. We find that the symbiont increases the mean and the variance in Ae. aegypti susceptibility to dengue infection. While higher mean susceptibility alone should lead to enhanced viral transmission, the increase in variance widens the basis for selection imposed by unexplored natural forces, such as mosquito pathogens, which need to be catalogued before net effects can be predicted.

**Results**

**Susceptibility distributions of mosquitoes to dengue viruses.** Figure 1 shows the estimation of susceptibility distributions for populations of Ae. aegypti (Wolb+ denoting non-carriers of Wolbachia, and Wolb− the carriers). Rio de Janeiro mosquitoes were challenged by injection with dilutions of a serotype 1 virus previously isolated from a patient and amplified, whereas in Ho Chi Minh City the adopted procedure was feeding on viremic blood from multiple infected patients. Either case provided suitable data for fitting dose-response models as described in Methods section. Figure 1a, b shows the data and model fittings, which resulted in the susceptibility distributions plotted in Fig. 1c, d. Models with gamma-distributed susceptibilities performed better than their homogeneous counterparts, according to model selection criteria, except in the case of Brazilian Wolb− challenged by injection. Estimated parameters are shown in Table 1 and model selection was performed by deviance information criterion (DIC) (Supplementary Tables 1 and 2). Wolbachia consistently increased the mean susceptibility of mosquitoes to dengue viruses (by average factors of 6.9 and 1.5 in the experiments of Brazil and Vietnam, respectively), and in addition it increased the variance-to-mean ratio in the trait (to 20.83 in Brazil, and 7.2 in Vietnam). The remainder of this paper addresses the implications of these findings on the prospects for Wolbachia to invade Ae. aegypti populations and eliminate dengue as a human disease.

**Wolbachia invasion dynamics.** The first reports of Wolbachia interfering with host susceptibility to pathogens are dated no more than a decade ago, and association studies suggest that general mechanisms modify susceptibility to broad spectra of viruses to similar extent. The advent of sequence-independent techniques, based on small RNAs produced by the host, to characterize insect viruses, opens exciting opportunities to study the depth of such associations. In anticipation, a suite of unspecified pathogens capable to infect mosquitoes is contemplated in our models by a parameter λM, whose value can be set to any positive real number or zero, and individual susceptibility to the pathogen ensemble is treated as being perfectly correlated with susceptibility to dengue viruses. Moreover, these pathogens are assumed to be host generalists (i.e., capable to infect other host species), and thus their abundance is not determined by the specific interactions with Ae. aegypti. This enables an initial exploration of the expected impacts of Wolbachia-induced variation in mosquito susceptibility to viruses, which may be refined if richer pathogen-specific datasets become available. In a similar vein, possible correlations or trade-offs between viral susceptibility and other life-history traits are not contemplated at this stage.

Figure 2 addresses the invasiveness of mosquito populations carrying Wolbachia, upon releases in Rio de Janeiro and Ho Chi Minh City, accounting for the susceptibility distributions estimated here in conjunction with various demographic parameters estimated previously (Table 2), as a function of mosquito pathogen abundance, λM. The solid black curve in Fig. 2a, b depicts the threshold frequency that Wolbachia carriers must attain in the population for their invasion to be predicted, despite specific costs associated with the symbiosis, such as measured reductions in fecundity and lifespan. Population densities where Wolbachia is absent or fixed are also shown for completeness. The figure reveals how mosquito pathogens, which act as a force of selection upon susceptibility distributions, are expected to facilitate Wolbachia invasions due to the higher variance in the trait among carriers.

The dotted lines in Fig. 2a, b shows how the invasion threshold would be expected to increase with exposure to mosquito pathogens given the increases in mean susceptibility attributed to Wolbachia in our analysis of experimental data, if the changes in variance had been ignored. When entire distributions are considered, the greater variance among carriers reverses the trend and, if pathogen exposure is sufficiently high, the threshold may eventually vanish. This is due to cohort selection, which is disabled in mean field
approximations. When a population with a given susceptibility distribution is released from the laboratory to some pathogen-richer environment, pathogen pressure determines the profile that will be effectively established. Since pathogens affect predominantly those individuals who are more susceptible, mean susceptibility decays with time since release (Fig. 2c, d), facilitating invasion. Everything else being the same, invasion thresholds are lower for populations with higher variance. In this process, variance-to-mean ratios also decrease, which is consistent with the notion that the lower variances exhibited by resident (compared to introduced) mosquitoes are a natural consequence of previous exposure to selection.

The earlier releases of *Ae. aegypti* mosquitoes transinfected with wMel occurred in Northern Queensland, Australia—first in isolated communities (Gordonvale and Yorkeys Knob, 2011)12, and later in a city (Cairns, 2013)23—where ongoing monitoring provides growing datasets to test models. In these settings, the invasion threshold was estimated to be in the range 0.2–0.35. According to our Fig. 2a, b, thresholds in this range are expected for Rio de Janeiro and Ho Chi Minh City Vietnam when pathogen pressure is included ($\lambda_M$ around 0.3), but would appear too high otherwise (that is, if $\lambda_M = 0$). Also notable is the lower amplitude of seasonal fluctuations observed in *Wolbachia*-carrying populations (relative to non-carriers) within release zones23, which would be predicted by cohort selection on a population with higher variance in susceptibility (Fig. 3e, k).

### Dengue transmission dynamics

Figure 3 shows the results of further increasing the model with dengue transmission in a human population24, calibrating the outputs prior to *Wolbachia* releases to dengue notification data in the two study cities, and then simulating releases in 201725,26. A 4-serotype model was necessary to meet the higher incidences observed in Brazil (Supplementary Fig. 1). In Rio de Janeiro, where *Wolbachia* was estimated to increase mean susceptibility by a factor of 6.9, dengue transmission would be slightly increased by *Wolbachia* fixation in the absence of selection (Fig. 3a). However, when selection by pathogens is present, as expected, a population of *Wolbachia*-carriers with the same characteristic can reduce dengue transmission substantially, by an extent that increases with the intensity of pathogen exposure. In Ho Chi Minh City, the predictions are less extreme because *Wolbachia*-mediated increases in the mean and variance of the susceptibility distribution are smaller.

In the absence of pathogens, we expect a slight reduction in dengue transmission (Fig. 3g), despite the increase in mosquito mean susceptibility to infection to a factor of 1.5. This is due to a reduction in infectivity ($\rho_w/\rho_0 = 0.45$), inferred as the proportion of infected mosquitoes that had virus in the salivary glands4. The same factor was adopted for the Brazil simulations, although shown by itself insufficient to reduce transmission in that setting. The effects of *Wolbachia* on the reproduction number, $R_0$, were calculated for each scenario, resulting in factors in the ranges 0.30–4.0 for Brazil and 0.42–0.88 for Vietnam, with the amplitude of the range attributed to the intensity of selection imposed by mosquito pathogens.

The results in Fig. 3 were obtained considering a human population with heterogeneous risk of acquiring and transmitting dengue infection. Little is known about risk distributions for acquiring dengue in humans, although estimates for other diseases indicate variance-to-mean ratios in the 4–20 range27,28. Here we have adopted the conservative value of 4 and show, for comparison, the corresponding results generated under variance-to-mean ratios of 20 (Supplementary Fig. 2) and 0 (Supplementary Fig. 3). Reductions in dengue transmission appear greater under more homogeneous human population models, although this would not have been captured by $R_0$ ratios alone. In Vietnam,
elimination of dengue is predicted under either sufficiently low heterogeneity in the human population, or sufficiently high-cohort selection in the mosquito population, whereas in Brazil, where baseline incidence is higher, stable elimination is not predicted in any of the scenarios contemplated here.

**Discussion**

Analysis of two independently generated sets of dose-infectivity curves, shows that *Wolbachia* consistently increases the mean and the variance in *Ae. aegypti* mosquito susceptibility to dengue viruses. These effects appear greater in Brazil, where mosquitoes were challenged by injection, than in Vietnam, where challenges were by ingestion of viremic blood from infected patients. The finding that *Wolbachia* increases variation in susceptibility to a virus is compatible with a study previously carried out in *Drosophila melanogaster* flies and demands devoted research.

*Wolbachia* has often been found to protect hosts against pathogens, but not always. There is a cumulative number of studies, both in natural and artificial *Wolbachia*-host systems, which report that *Wolbachia* enhances or has no noticeable effect on specific pathogens. To what extent these discrepancies are real or methodological is to be inquired. In the

Table 1 Parameter estimates from dose-response model fitting to experimental data

| Parameter | $p$ | $\alpha_{Wolb^+}$ | $\theta_{Wolb^+}$ | $\alpha_{Wolb^-}$ | $\theta_{Wolb^-}$ |
|-----------|-----|-------------------|-------------------|-------------------|-------------------|
| Brazil    |     |                   |                   |                   |                   |
| MAP       | $1.149 \times 10^{-7}$ | 0.3332            | 20.77             |                   |                   |
| Median    | $1.066 \times 10^{-7}$ | 0.3465            | 26.54             |                   |                   |
| 95% CI    | $5.720 \times 10^{-8}$ | 0.1547            | 2.566             |                   |                   |
|           | $1.837 \times 10^{-7}$ | 0.8907            | 447.0             |                   |                   |
| Vietnam   |     |                   |                   |                   |                   |
| MAP       | 0.3602 | 347.1             | 910.3             |                   |                   |
| Median    | 0.3652 | 401.9             | 944.5             |                   |                   |
| 95% CI    | 0.3045 | 235.4             | 437.7             |                   |                   |
|           | 0.4366 | 761.4             | 910.3             |                   |                   |

MAP denotes maximum a posterior probability. In Brazil, $p$ is the probability of infection for a *Wolb^+* mosquito per unit of viral challenge (TCID$_{50}$), and $\alpha_{Wolb^+}$, $\theta_{Wolb^+}$ are the shape and scale parameters, respectively, of the gamma distribution determining the susceptibility factors of *Wolb^+* mosquitoes in relation to *Wolb^−* (i.e., with respect to $p$). In Vietnam $p_{aux} = 10^{-6}$ (per log$_{10}$ viral titre (RNA copies/ml)), was used as an auxiliary parameter, while $\alpha_{Wolb^−}$, $\theta_{Wolb^−}$ and $\alpha_{Wolb^+}$, $\theta_{Wolb^+}$ define the distributions of susceptibility of *Wolb^−* and *Wolb^+* mosquitoes, respectively, in relation to $p_{aux}$.

**Fig. 2** Threshold for *Wolbachia* invasion and cohort selection on susceptibility. **a, b** Invasion threshold for *Wolbachia* carriers over a range of forces of selection by mosquito pathogens, obtained by introducing the susceptibility distributions estimated in Fig. 1 in a population dynamic model described in Methods section (solid back curves). Dotted lines show the results of the same calculation if only the means had been used to describe the susceptibility of each population. Other model outputs are the population sizes of *Wolbachia*-free (*Wolb^−*, in blue) and *Wolbachia*-carrier (*Wolb^+*, in green) populations at fixation, in Rio de Janeiro, Brazil (a) and Ho Chi Minh City, Vietnam (b). **c, d** Effects of cohort selection on the mean susceptibility of each population once equilibrium has been reached under a range of selection forces. The insets illustrate how mean susceptibilities develop over time, from the moment populations are released until equilibria are established, under one specific force of selection ($\lambda_M = 0.3$). The changes in mean susceptibility depicted in the insets are accompanied by reductions in variance. Variance-to-mean ratios in *Wolb^+* populations changed from 20.83 to 10.10 in Brazil (c), and from 7.200 to 0.7715 in Vietnam (d).
light of our findings, a more comprehensive analysis of all available data is warranted. First, we convey that no conclusions can be drawn from standard single-dose experimental challenge designs. Second, we demonstrate that inclusive dose-response analyses may lead to results which contradict those obtained by averaging single-dose findings. Third, we find that by consistently increasing variation in host susceptibility to viruses, Wolbachia transfection creates a population whose effective mean susceptibility is highly sensitive to natural selective pressures operating in specific release sites. Given that Wolbachia appears to modify host susceptibility to a broad spectrum of pathogens,7,17, reliable predictions of invasiveness and vectorial capacity of transfected mosquitoes require an informed account of natural mosquito pathogens and their interplay with Wolbachia.

Having exposed the fundamental roles of individual variation in host (mosquito or human) susceptibility, or exposure, to infections in leveraging population measures, the adoption of homogeneous models, for assessing and predicting the response to interventions, is no longer an option. A dose-response experimental design analogous to that performed here has recently been adopted to assess a vaccine against a virus of rainbow trout33, while suitable study designs on natural endemicity gradients can enable the estimation of essential heterogeneity parameters from field settings, just like dose-response experimental designs do in the laboratory.

**Methods**

**Data preparation.** We reanalyse two previously published datasets, where *Aedes aegypti* mosquitoes were challenged with doses of dengue virus spanning a suitable range, and viral titres were subsequently measured to determine infection status. The experimental challenge procedures were performed on two mosquito populations: carrying a wMel Wolbachia strain (Wolb); and not carrying any Wolbachia (Wolb+). In one of the experiments, mosquitoes from Rio de Janeiro, Brazil, were challenged by injection, while in the other, mosquitoes from Ho Chi Minh City, Vietnam, were challenged by feeding on viremic blood from infected patients. The proportions of mosquitoes with detectable virus were assessed at various days after infection (range 3–14 in Brazil, and 7–18 in Vietnam), and data were formatted in a dose-response manner, with dose referring to challenge and response representing probability of infection.

**Dose-response model.** Adopting established formulations, we denote by *d* the viral challenge dose (measured in units of 50% tissue culture infective dose [TCID50] in Brazil, and log10 viral titre [RNA copies/ml] in Vietnam) and *p* a measure of infectivity to a mosquito host per unit of viral challenge; the number of infecting units per host is assumed to follow a Poisson distribution with mean *pd*. When all hosts are equally susceptible, the probability of a host remaining uninfected after viral challenge is the zeroth term of the distribution, leading to a probability of infection *ninf* = 1 − *e−pd*. When individual hosts vary in their susceptibility to infection, we consider the infectivity of each unit of challenge to vary between hosts according to a gamma distribution *q*(x) = *xα−1*e−*x/θα*/θα, where *α* and *θ* are the shape and scale parameters, respectively, resulting in the modified dose-response model *ninf* = 1 − ∫ 1 − *e−pdq*(x)dx, or its closed form *ninf* = 1 − [1/(1 + θαpd)]α, which is obtained by Laplace transform. The factors *x* are dimensionless measures of relative susceptibility between hosts.

**Susceptibility distribution estimation.** Dose-response models are fitted to the experimental data using a Bayesian Markov Chain Monte Carlo (MCMC) based method, implemented in Python programming language with PyMC package, assuming uniform priors, to estimate distributions of susceptibility of mosquito populations to dengue viruses. The likelihood of the parameters given the data is computed using binomial distributions written for a given dose as

\[
P(\mathbf{K} | N, d, p) = \binom{N}{k} d^k (1 - d)^{N-k},
\]

where *N* is the number of mosquitoes being challenged, *r* is the probability of obtaining a successful response from each challenge (i.e., successful infection by the virus), *k* is a function of *p* and *d*, according to either homogeneous (*ninf*) or heterogeneous (*ninf*) model formulations given above, and *k* is the observed number of infected mosquitoes (i.e., with detectable viral titres). The fit of the homogeneous model to data across *D* doses gives an estimate of parameter *p*, whose log-likelihood is given by a sum of binomial distributions

\[
P(\mathbf{K} | \mathbf{N}, d) = \sum_{i=1}^{D} \log \left( \binom{N_i}{k_i} r_i^k (1 - r_i)^{N_i-k_i} \right),
\]

where *K*, *N*, and *d* are vectors composed of *k*, *N*, and *d*, respectively, and *r* = 1 − *e−pd*. For fitting the heterogeneous model, the log-likelihood is given by

\[
P(\mathbf{a}, \theta | \mathbf{K}, \mathbf{N}, d, p) = \sum_{i=1}^{D} \log \left( \binom{N_i}{k_i} \theta^k (1 - r_i)^{N_i-k_i} \right),
\]

where *r* = 1 − [1/(1 + θninf*pd*)]α, and *ninf* is treated as a fixed auxiliary parameter.

Brazilian and Vietnamese studies are analysed separately. Model formulations where the susceptibility of individual mosquitoes is considered homogeneous or gamma-distributed are both fitted to Wolb+ data. The deviance information criterion (DIC) is applied to select between the two formulations, favouring homogeneous in Brazil, and heterogeneous in Vietnam (adopting *p*aux = 1 in Supplementary Table 1, and *p*aux = 10−8 in Supplementary Table 2).
**Fig. 3** Projected impact of *Wolbachia* on dengue transmission in human populations. Colours indicate whether transmission is by *Wolbachia*-free (Wolb−, in blue) or *Wolbachia*-carrier (Wolb+, in green) mosquitoes. Left panels show dengue incidence versus transmission coefficient plotted from equilibrium solutions of a 4-serotype dengue model (Methods section) on human populations with heterogeneous risk (variance-to-mean ratio of 4): a, d mosquitoes parameterized from experiments in Brazil and released under different forces of selection by mosquito pathogens; a λM = 0; d λM = 0.3. g, j same as a, d, for Vietnam. Equilibrium curves adopted mean proportions of infected mosquitoes with detectable virus in the salivary glands§, and shaded areas represent lower and upper bounds encountered when mosquitoes were stratified by challenge dose. Dotted lines mark dengue incidence in Rio de Janeiro, and Ho Chi Minh City, averaged over a 4-year period and scaled by an expansion factor of 5). Parameter values: β = 0.59 (c), β = 0.74 (f), β = 0.13 (l), β = 0.17 (f); A = 0.25, B = 0.25 (b, e, f, t); A = 0.05, B = 0.1 (h, i, k, l).

The selected model is then extended with a gamma-distributed susceptibility for *Wolbachia* invasion and dengue transmission.

### Wolbachia invasion model

The conditions for *Wolbachia* invasion are assessed using a model that describes the population dynamics of two interbreeding *Ae. aegypti* populations: a resident *Wolbachia*-free population, U, and an introduced *Wolbachia*-carrier population, W. *Wolbachia* is considered to modify the longevity of its carriers by a factor $s_l$, the fecundity by a factor $s_f$, and to reduce the reproduction of U individuals via cytoplasmic incompatibility by a factor $s_i$. Moreover, *Wolbachia* is assumed to modify the general susceptibility to mosquito pathogens by a factor that is positively correlated with that estimated for dengue viruses. The population dynamics is thus given by the following system of differential equations:

$$\frac{du(x)}{dt} = q_u(x)uU \left( \frac{U + W(1 - s_i)}{M} \right) - (b + kM)s_u(x) - x\lambda_M u(x).$$ \hspace{1cm} (4)

$$\frac{dw(x)}{dt} = q_w(x)wW \left( \frac{1 - s_i}{1 - s_j} + kM \right)w(x) - x\lambda_M w(x).$$ \hspace{1cm} (5)

where $q_u(x)$ and $q_w(x)$ are the susceptibility distributions of populations U and W, respectively, $U = \int u(x)dx$ and $W = \int w(x)dx$ represent the total Wolb− and Wolb+ populations and $M = U + W$ the total mosquito population. For parameter definitions and values, see Table 2.
This system of differential equations has been modified from previous studies\(^{9,11}\), and maintains the essential qualitative properties. It has three non-trivial equilibrium solutions: two stable and one unstable. The stable equilibria are referred to as the pre- and post-invasion equilibria, where either the \(U\) or the \(W\) population, respectively, has reached fixation. A third equilibrium can be found which accommodates both populations. Being unstable, however, any deviation will lead to fixation of one of the other population. Therefore, in practice, such a state does not persist, but instead sets a threshold frequency of \(Wolbachia\)-carriers necessary for invasion\(^{19}\)—the invasion threshold, \(p\). Spatial dimensions may be included in the model and are expected to slow down local invasions due to reintroductions of non-carriers from neighbouring areas\(^{3}\). However, this is beyond the scope of this study.

Numerical solutions were obtained in Matlab by discretizing each susceptibility distribution into 100 parts and simulating the resulting system of 200 ordinary differential equations.

**Dengue transmission model.** To assess the effectiveness of \(Wolbachia\) in modifying the vectorial capacity of \(Ae. aegypti\), we build a 4-serotype susceptible-infected-recovered (SIR) model for the human population, with transmission between humans mediated by mosquitoes that may carry or not the \(Wolbachia\) symbiont. Denoting by \(V = \{1, 2, 3, 4\}\) the set of dengue serotypes, the model for a homogenous host population is formulated in set notation\(^{36}\) as

\[
\begin{align*}
\frac{dS_h}{dt} &= \rho H \left( 1 - \beta \left( \rho_l \sum_{i=1}^{N} U_l + \rho_w \sum_{i=1}^{N} W_l \right) \right) S_h - \mu S_h, \\
\frac{dS_f}{dt} &= \rho F \left( 1 - \beta \left( \rho_l \sum_{i=1}^{N} U_t + \rho_w \sum_{i=1}^{N} W_t \right) \right) S_f - \mu F S_f, \\
\frac{dS_i}{dt} &= \beta \left( \rho_l \sum_{i=1}^{N} U_l + \rho_w \sum_{i=1}^{N} W_l \right) S_i - \mu I, \quad \text{for} \, \beta \, \mathcal{J} \subseteq \mathcal{V}, \\
\frac{dS_t}{dt} &= \beta \left( \rho_l \sum_{i=1}^{N} U_t + \rho_w \sum_{i=1}^{N} W_t \right) S_t - \mu I, \quad \text{for} \, \beta \, \mathcal{J} \subseteq \mathcal{V}.
\end{align*}
\]

(6)

and

\[
\begin{align*}
\frac{dS_h}{dt} &= \beta \left( \rho_l \sum_{i=1}^{N} U_l + \rho_w \sum_{i=1}^{N} W_l \right) S_h - \mu S_h, \\
\frac{dS_f}{dt} &= \beta \left( \rho_l \sum_{i=1}^{N} U_t + \rho_w \sum_{i=1}^{N} W_t \right) S_f - \mu F S_f, \\
\frac{dS_i}{dt} &= \beta \left( \rho_l \sum_{i=1}^{N} U_l + \rho_w \sum_{i=1}^{N} W_l \right) S_i - \mu I, \\
\frac{dS_t}{dt} &= \beta \left( \rho_l \sum_{i=1}^{N} U_t + \rho_w \sum_{i=1}^{N} W_t \right) S_t - \mu I, \\
\end{align*}
\]

(7)

and

\[
\begin{align*}
\frac{d(\lambda_i u_i)}{dt} &= \gamma \int_{\mathcal{J} \cap \mathcal{V}} \left( \phi \, \mathcal{J} + \phi \, \mathcal{J} \right) \, dx, \\
\frac{d(\lambda_i w_i)}{dt} &= \gamma \int_{\mathcal{J} \cap \mathcal{V}} \left( \phi \, \mathcal{J} + \phi \, \mathcal{J} \right) \, dx, \\
\end{align*}
\]

(8)

Finally, we note that the model was simulated to generate time series solutions of the 4-serotype model, compared with lower dimensional versions generated with less serotypes (3, 2 or 1). The obtained incidence levels for Rio de Janeiro and Ho Chi Minh City are also plotted, showing, among other things, the need to adopt the full 4-serotype model to meet incidences as high as those in Brazil.

**Dengue incidence data.** Dengue incidence was approximated from case notifications in Rio de Janeiro, Brazil (2010–2013) and Ho Chi Minh City, Vietnam (National Institute of Hygiene and Epidemiology (1995–2010) and Project Tycho, www.tycho.pitt.edu/dev/pnas/index.php). Based on official population counts, we adopted the approximate values of 6 million for Rio de Janeiro (Census Bureau, Brazilian Institute of Geography and Statistics (2010–2013) and 8 million for Ho Chi Minh City (General Statistics Office of Vietnam (1995–2010))).

**Model calibration.** Model calibration was performed for Rio de Janeiro, Brazil, and Ho Chi Minh City, Vietnam, by calculating the endemic steady state as a function of the transmission coefficient \(\beta\) and adjusting this parameter to the annual incidence averaged over a period of 4 years (2010–2013 for Rio de Janeiro; 2007–2010 for Ho Chi Minh City). An expansion factor of 5 was applied to the official notifications to account for unapparent cases, as recommended by published studies in both countries\(^{4,12}\).

**Time series model.** The model described was simulated to generate time series prior to \(Wolbachia\) introduction, to serve as a basis for projections of expected impact after releases occur. Simulations were performed with seasonal forcing on the transmission coefficient:

\[
\beta(t) = \beta_0 \left[ 1 - B \cos \left( \frac{2 \pi t}{365} \right) \right],
\]

(18)

where \(\beta_0\) is the average transmission coefficient estimated for each scenario by the steady-state calibration above, and \(B\) is the amplitude of seasonal forcing on transmissibility, as well as on mosquito birth rates.

\[
a(t) = a_0 \left[ 1 - A \cos \left( \frac{2 \pi t}{365} \right) \right],
\]

(19)

where \(a\) is the birth rate as in Table 2 and \(A\) is the amplitude of seasonal forcing on birth rate.

**Data availability.** All data analysed in this paper are available from the cited publications Ferguson et al.\(^9\) and Souto-Maior et al.\(^14\).

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