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Using *Wolbachia* for dengue control: insights from modelling

Ilaria Dorigatti^{1,*}, Clare McCormack^{1,*}, Gemma Nedjati-Gilani^{1,*}, and Neil M Ferguson^{1,+}

¹MRC Centre for Outbreak Analysis and Modelling, Department of Infectious Disease Epidemiology, Imperial College London, London W2 1PG

Abstract

Dengue is the most common arboviral infection of humans, responsible for a substantial disease burden across the tropics. Traditional insecticide-based vector control programmes have limited effectiveness and the one licensed vaccine has a complex and imperfect efficacy profile. Strains of the bacterium *Wolbachia* deliberately introduced into *Aedes aegypti* have been shown to be able to spread to high frequencies in mosquito populations in release trials, and mosquitoes infected with these strains show markedly reduced vector competence. Thus, *Wolbachia* represents an exciting potential new form of biocontrol for arboviral diseases, including dengue. Here we review how mathematical models give insight into the dynamics of the spread of *Wolbachia*, its potential impact on dengue transmission and discuss remaining evaluation and development challenges.

The need for better dengue control

Dengue is an acute systemic disease caused by one of four genetically related but antigenically distinct serotypes (DENV1-4), and is the most prevalent human arboviral infection globally, with almost half of the world's population at risk of infection [1,2]. Since 1943, when the first dengue virus serotype was isolated in Japan, dengue transmission has followed the rapid geographical expansion of its most competent vector, the *Aedes aegypti* mosquito, causing a substantial and ever-increasing public health and economic burden throughout the tropics [3–5]. In the absence of specific antiviral treatment and, until recently, a vaccine against dengue, disease control has long relied on the suppression of mosquito populations through environmental management, biological control and chemical interventions [6]. If rigorously applied, these traditional forms of control can be effective in reducing the size of mosquito populations and decreasing the burden of dengue at small spatial scales in the short term. Nonetheless, there is increasing evidence that such measures are both ineffective and unsustainable in the long term, with mosquito populations returning to pre-treatment levels after control interventions cease [7]. In addition, the implementation of large scale insecticide-based programmes gives rise to ecological and environmental concerns, including promoting the evolution and spread of insecticide resistance, which

⁺Correspondence: neil.ferguson@imperial.ac.uk (N.M. Ferguson).

^{*}Equal contributions

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appears to be emerging in several countries, thus causing further detrimental effects on dengue control [8]. Several dengue vaccines are currently in development [9,10] and the first dengue vaccine (Dengvaxia), developed by Sanofi-Pasteur, was licensed in 2015. However, Dengvaxia has a complex efficacy profile, with questions around its long-term safety and efficacy in dengue-seronegative recipients [11–13]. Vaccination is therefore currently recommended exclusively in moderate to high-transmission settings [14], where 80% vaccination coverage in 9-year olds has been predicted to reduce symptomatic disease and hospitalisations by 20–30% at most [15,16]. Hence, there remains an urgent need for new interventions which have the potential to substantially reduce dengue transmission and disease burden across a wide range of settings.

Two new approaches have been recently tested in field trials. The first is based on the release of genetically modified male mosquitoes that carry a dominant lethal gene. This strategy has been shown to be able to suppress mosquito populations, but requires the continuous release of large numbers of transgenic mosquitoes for several months [17]. However, in the absence of a gene drive system, questions remain about the sustainability of this intervention, as the migration of unmodified mosquitoes from neighbouring areas following local suppression of a mosquito population requires releases to be continued indefinitely, albeit at lower levels than during initial suppression [18].

The second approach involves the release of mosquitoes transfected with the vertically transmitted intracellular bacterium *Wolbachia*. *Wolbachia* has been shown to be able to establish itself in mosquito populations [19] and to suppress arbovirus replication in mosquitoes, and so is a potentially promising means to controlling dengue transmission in endemic settings [20]. Here we review the milestones in the development of *Wolbachia* transinfection in *Aedes aegypti* as a tool for dengue control, with a focus on the mathematical models developed to date to study the invasion dynamics of *Wolbachia* and its impact on dengue transmission. We discuss the uncertainties and challenges that lie ahead for the use of *Wolbachia* as a potential new form of biocontrol for dengue.

***Wolbachia* transinfection**

Wolbachia is an endosymbiotic intracellular bacterium that is naturally present in about 60% of all insect species [21,22], including some mosquitoes. Mosquito species known to be naturally infected with *Wolbachia* include *Culex pipiens* [23] and *Aedes albopictus* [24], one of the vectors for dengue virus, but not *Aedes aegypti*, the primary vector for this virus.

Wolbachia is transmitted maternally and alters the reproductive phenotype of infected insects to give the bacteria a reproductive advantage relative to uninfected insects. The reproductive phenotype expressed depends on both the insect species and *Wolbachia* strain, and can result in feminisation, male killing, parthenogenesis or, most commonly, cytoplasmic incompatibility [21].

Cytoplasmic incompatibility (CI), the phenotype expressed by the *Wolbachia* strains developed for *Aedes aegypti* transfection, renders eggs laid as the result of an uninfected female and infected male cross unviable, whereas *Wolbachia*-infected females lay viable

Wolbachia-positive eggs regardless of the infection status of the male (Figure 1) [21]. However, depending on the *Wolbachia* strain, infection can impose additional fitness costs on infected insects, such as increased adult mortality [25], reduced fecundity [25], prolonged egg and larval development time [26], and reduced survival of desiccated eggs [27]. This leads to a trade-off between the reproductive advantage bestowed on *Wolbachia*-infected mosquitoes (relative to wild type) by CI and the fitness costs of infection, resulting in a frequency dependent invasion threshold which must be exceeded to ensure fixation of *Wolbachia* in a wild population [28]. This threshold will also depend on the degree of vertical transmission among *Wolbachia*-infected mosquitoes [29,30].

Although not naturally present in *Aedes aegypti*, *Wolbachia* was introduced into the species artificially via transinfection. This was demonstrated for the first time in 2005 by Xi et al [31], when they isolated *Wolbachia*-containing cytoplasm from *Aedes albopictus* eggs, which are naturally infected with both *wAlbA* and *wAlbB* strains, and used microinjection to transfer the bacteria into *Aedes aegypti* eggs, resulting in two stable *wAlbB*-infected *Aedes aegypti* lines. Laboratory experiments showed almost perfect maternal transmission and CI in these lines. The initial rationale for introducing *Wolbachia* into *Aedes aegypti* mosquitoes was to demonstrate that CI could be used as a drive mechanism to introduce transgenes into an *Aedes aegypti* population, rather than make use of the properties of *Wolbachia* itself as a dengue control measure. However, based on the observation that *Drosophila melanogaster* fruit flies infected with the virulent *wMelPop* strain have increased adult mortality [32], it was hypothesised that the translation of these fitness costs to *Aedes aegypti* might reduce population density and the mean lifespan of an infected mosquito (possibly to below the extrinsic incubation period of dengue virus), thus greatly reducing their potential to transmit the virus onwards after an infectious blood meal [33].

In 2009, transinfection of the *wMelPop* *Wolbachia* strain from *Drosophila melanogaster* to *Aedes aegypti* eggs was demonstrated by McMeniman et al [33]. Experiments showed a 19% reduction in fecundity and an over 50% increase in adult mortality due to *wMelPop* infection in *Aedes aegypti*. However, while initial cage invasion experiments [34] showed that with a high initial release fraction, *wMelPop*-infected mosquitoes can invade wild-type populations, the high fitness costs raised concerns about the viability of establishing *wMelPop* in the field. Thus in parallel to the work on *wMelPop*, a second less virulent *Wolbachia* strain, *wMel* [35], was also transinfected from *Drosophila* to *Aedes aegypti* [34], with experiments showing almost perfect CI and maternal transmission, but much lower (non-significant) fecundity and mortality costs of only 10% (vs 50% with *wMelPop*).

More unexpectedly, both *wMel* and *wMelPop* infection was demonstrated to dramatically reduce vector competence; *wMelPop*-infected mosquitoes fed with blood spiked with DENV2 virus showed no evidence of dengue virus in their salivary glands after 14 days, compared with 81% of wild-type mosquitoes [34]. Virus replication was also dramatically reduced in *wMel*-infected *Aedes aegypti*, albeit not as completely as for *wMelPop*, with 4.2% of mosquitoes showing DENV2 virus in their saliva 14 days after blood-feeding [34].

However, further examination revealed that each pooled sample of saliva showing DENV2 virus after 14 days contained saliva from *wMel*-uninfected mosquitoes, suggesting that imperfect maternal transmission may have contributed to these results [34].

Joubert et al. [36] have recently demonstrated that *Wolbachia* superinfection is possible, by generating a line of *Aedes aegypti* infected with both *wMel* and *wAlbB* and showing that the superinfection generates unidirectional CI when crossed to each single infected parental line. No significant differences in fitness were observed between the superinfected line and either single infected parental line, suggesting that the superinfection has the potential to replace single constituent *Wolbachia* strains already present in a mosquito population. Furthermore, upon challenge with blood meals from viremic dengue patients, the superinfected line was observed to also reduce dengue virus replication notably more efficiently than the parental *wMel* line [36].

From the laboratory to field releases

Cage experiments demonstrated that both *wMelPop* and *wMel* infected mosquitoes could invade wild-type populations and achieve high frequencies [33,34]. However, for *Wolbachia* to be a viable dengue control measure, it was necessary to demonstrate invasion was possible in field conditions. Field releases were also needed to understand the real-world dynamics of invasion and to optimise release strategies. Initial studies in two small communities near Cairns, Australia in January 2011 [19] involved the release of almost 300,000 *wMel*-infected mosquitoes in total over a period of 10 weeks. Counts of *wMel*-infected and wild type mosquitoes from traps collected 5 weeks after the end of releases indicated that the frequency of *Wolbachia* in the local *Aedes aegypti* population had reached 90%. Further analysis of the data [37] has also allowed researchers to explore the spatial patterns of *Wolbachia* abundance during the releases, including the effect of dwelling type and land cover on the proportion of *Wolbachia*-positive *Aedes aegypti* mosquitoes, which could guide the strategy of future releases. Follow-up studies indicated that *Wolbachia* infection is stable and persistent, with no measurable drift in maternal transmission or CI, and with long-term average infection frequencies over 94.0% [38]. Furthermore, they have indicated that *Wolbachia* infection continues to reduce vector competence following establishment in wild-type populations [39].

Field releases of *wMelPop*-infected *Aedes aegypti* were conducted near Cairns, Australia and Tri Nguyen, Vietnam [40]. In Australia, 15 releases took place in the wet season over a 4 month period starting in January 2012, with 6 additional releases carried out during the dry season in one location. In Vietnam, pupae (instead of adults) were released over a period of 23 weeks starting in April 2013, following an active *Aedes aegypti* suppression campaign. In all locations, the frequency of *Wolbachia* as measured from trapping data exceeded 90% by the end of the release period; however, by the end of the monitoring period (approximately 40–60 weeks post-initial release), the frequency had dropped to less than 20%, supporting the hypothesis that the fitness costs of *wMelPop* are too severe to be self-sustaining in wild populations. Following on from these releases, *wMel*-infected *Aedes aegypti* have been released into and successfully invaded all three locations within a shorter release window [40].

So far, all field releases have focused on entomological endpoints to establish the sustainability of *wMel* in wild *Aedes aegypti* populations. However, in order to demonstrate its viability as a dengue control measure, large scale releases are now occurring in Indonesia (<https://clinicaltrials.gov/ct2/show/NCT03055585>), Australia, Colombia and Brazil [41], Vietnam (<http://www.eliminatedengue.com/vn/progress>).

While in this article we focus on the use of *Wolbachia* as a population replacement strategy (i.e. permanently establishing *Wolbachia* infection in the *Aedes aegypti* population), the CI phenotype *Wolbachia* infection bestows can also be exploited as an alternative to genetic modification or radiation based sterilisation for population suppression strategies relying on the release of sterile males. The Singaporean National Environment Agency conducted small-scale field releases of *Wolbachia*-infected male mosquitoes, beginning in Singapore in 2016 (<http://www.nea.gov.sg/public-health/environmental-public-health-research/wolbachia-technology/project-wolbachia-singapore/wolbachia-aedes-small-scale-field-study>), to test the dispersal, longevity and competitiveness of *Wolbachia*-carrying mosquitoes compared to male *Aedes aegypti* in an urban setting. In addition, two US companies, MosquitoMate (<http://mosquitomate.com>) and Verily (<https://verily.com>) partnered to conduct a large scale population suppression trial using this approach in Florida in 2017. Similar approaches are also being adopted for *Aedes albopictus* suppression in Guangzhou, China (<https://www.reuters.com/article/us-china-health-mosquitoes/chinas-mosquito-factory-aims-to-wipe-out-zika-other-diseases-idUSKCN10D0MV>). However, a challenge with using *Wolbachia* as a sterile male technology is the need for extremely accurate sex-sorting of insects prior to release, since the accidental release of even a small proportion of *Wolbachia*-infected females poses the risk of turning a population suppression strategy into a population replacement one; i.e. the establishment of breeding *Wolbachia*-infected females in the area where the wild-type population has been suppressed by the male releases.

Invasion dynamics of *Wolbachia*

The successful establishment and spread of *Wolbachia* in any given host population is dependent on the trade-off between the fitness benefits and costs incurred by infection with the bacteria. As first illustrated by Caspari and Watson [42] using a discrete generation population genetic model, this trade-off results in bistable dynamics, where two stable equilibria exist; one where infection frequency is zero and one where there is a high proportion of infected individuals (Figure 1B). To reach the non-zero equilibrium, infection frequency must exceed a critical threshold value, determined by the trade-off between the relative reduction in fecundity of infected females and the intensity of CI [29,30,42–44]. Extensions of this model predicted that if *Wolbachia* infection reduces the lifespan of infected hosts, a higher initial frequency of infected individuals may be needed for successful invasion [45]. In addition, if maternal transmission is not perfect, the speed of invasion may be reduced [46] and co-existence is more likely, as uninfected individuals are continually introduced into the host population [29,30]. Furthermore, when overlapping-generations are incorporated into models, the critical threshold frequency is seen to depend on the growth rate and age-structure of the population, in addition to the fitness effects of *Wolbachia* on the population [44]. Stochastic effects may also play an important role in the invasion dynamics, most notably when population sizes are small [47,48], as initial infection

frequencies below the (deterministic) threshold frequency can lead to successful *Wolbachia* establishment [47] and conversely, frequencies above the threshold do not necessarily lead to establishment.

Although *Wolbachia* infection may establish locally, this does not necessarily guarantee spatial spread, as spread beyond the local environment depends on the initial infection frequency, the critical threshold frequency, the dispersal behaviour of the host population and features of the underlying environment [43,49–53]. Initial analysis by Turelli and Hoffmann showed that a critical frequency threshold of less than 0.5 is necessary for spatial spread to occur following local establishment [43]. Schraiber et al showed a similar result accounting for lifespan-shortening as well as fecundity reducing effects [54]. Other analysis showed that as the critical threshold approaches 0.5, wave speed slows dramatically, suggesting that, in practical terms, a critical threshold value of 0.35 or less is necessary for spatial spread [52,55]. The critical threshold value, along with the initial infection frequency and dispersal behaviour of the host population, determines the size of the release area necessary to initiate spatial spread, with larger release areas needed for higher threshold values (Figure 2A–B) [55]. Long-range dispersal, as compared with local (Gaussian) diffusion, is predicted to reduce both the size of the release area needed and the wave speed [52]. Environmental heterogeneities, such as sharp changes in population density or habitat quality, may also slow or halt invasion unless migration of infected individuals from neighbouring areas is sufficient to allow the critical threshold frequency to be exceeded at the local level [52,53,55,56].

Models which consider the life cycle and demographics of mosquito populations have offered further insight into the dynamics of *Wolbachia* spread. Rather than primarily focusing on frequency thresholds for infection spread, population dynamics models instead consider how factors such as density-dependent competition, seasonal changes in abundance and population size affect the spread of *Wolbachia* through mosquito populations. The degree of density-dependent competition during the larval stage of population growth is predicted to have a considerable impact on the dynamics observed, with larger numbers of infected mosquitoes needing to be released to initiate spatial spread in populations subject to high levels of competition (Figure 2C) [57–59]. Last, seasonal variation in mosquito abundance is expected to affect establishment, with releases performed early in the wet season to coincide with early stages of population growth more likely to succeed [60]. Successful establishment in field settings will therefore depend on both the number of releases [52,58,60] and the type of release [60].

Predicted effect of *Wolbachia* on dengue transmission

The release of *Wolbachia*-infected *Aedes aegypti* mosquitoes has the potential to decrease, and in some settings, eliminate dengue transmission via two mechanisms: (a) by reducing mosquito population density and/or lifespan; (b) by reducing mosquito competence to transmit dengue.

Estimating the likely impact of these two effects on dengue transmission in the field from experimental data poses challenges. Early assessments of impacts on vector competence [34]

used blood artificially spiked with virus and estimated almost complete suppression of viral replication in *Aedes aegypti* [61–63]. Such levels of suppression would be expected to achieve elimination of transmission of dengue in contexts where *wMel* is able to be established in the local *Aedes* population at close to 100% frequency, given the relatively low basic reproduction number (the average number of human infections generated by a typical human infection in an immunologically naïve population), R_0 , of dengue [64]. However recent, more realistic experiments used fresh blood from dengue patients [20] and found somewhat lower levels of viral suppression compared with the earlier work [20]. The *wMelPop* strain was still seen to confer profound resistance to dengue infection (leading to > 90% blocking of transmission), but *wMel*-infected (and wild-type) mosquitoes exhibited infection rates which depended on the level of viremia in the blood meal [20]. Artificial infection experiments have also shown the potential for *wMel* to partially suppress replication of a range of other arboviruses, including Chikungunya, Yellow Fever and Zika [61–63].

When suppression is partial and dependent on viral titre in blood meals, modelling is needed to predict the overall impact of *wMel* infection on transmission [20]. To assess the likely impact of *wMel* infection on dengue, Ferguson et al. coupled data on viral dynamics within the human host with those in the vector, with the aim of translating the laboratory results into estimates of the potential impact of *wMel* on the R_0 of the different dengue serotypes [20]. This analysis predicted that *wMel* infection would reduce R_0 by 66% for DENV1 and 75% for DENV2/3/4, and therefore argued that widespread release of *Aedes aegypti* infected with *wMel* could eliminate dengue in low-to-moderate transmission settings (i.e. where $R_0 < 3-4$) even if infection with *Wolbachia* had no fitness costs on mosquitoes.

Limited mathematical modelling has been undertaken to date on the likely impact of *Wolbachia* on arbovirus transmission dynamics. In theoretically focussed studies, Hancock et al. [60] examined the impact of *Wolbachia* on transmission of a malaria-like disease, while Hughes and Britton [65] examined the potential impact of a *Wolbachia* strain with perfect maternal transmission and CI on transmission of a single strain arbovirus. Both studies examined the invasion dynamics of *Wolbachia* and how the phenotypic parameters of *Wolbachia*, which affect transmission and baseline pathogen transmissibility, determined the overall impact of *Wolbachia* on disease transmission. Other research has used simplified compartmental models of dengue transmission to examine similar issues [66–69], including the potential impact of *Wolbachia* on dengue outbreaks in non-endemic settings [67].

None of the modelling studies yet undertaken to explore the impact of *Wolbachia* on dengue transmission have used realistic 4-strain disease models [15] or incorporated the latest estimated impacts of *Wolbachia* on the R_0 of dengue [20]. Here we therefore adapt a published model of dengue transmission used to assess the likely impact of the Sanofi dengue vaccine on dengue disease burden [15] to incorporate *wMel*-infected as well as wild-type mosquitoes. We assumed that *wMel* infection increased adult mosquito mortality by 5% and decreased fecundity by 15% relative to wild-type, and that CI was perfect [34]. We varied two other phenotypic parameters: (a) the ability of a mosquito to transmit dengue (i.e. infectiousness) induced by *wMel* infection (between 20% and 100%); (b) the efficiency of maternal transmission of *wMel* (90%–100%). All other parameters were as previous published [15].

The predicted impact of blanket releases and successful establishment of wMel in a large human population (i.e. where exposure to dengue outside the wMel treated area makes up a negligible fraction of all dengue transmission prior to release) on dengue transmission dynamics is shown in Figure 3 (model available freely on request). We choose a deliberately conservative scenario, with wMel only reducing mosquito infectiousness (for dengue) by 40%, and maternal transmission of wMel only having 90% efficiency. The latter assumption is pessimistic, but motivated by one recent report of loss of wMel infection in some *Aedes aegypti* samples collected in the field[70]. Under these assumptions, following introduction, wMel only reaches a frequency of 87% due to the imperfect maternal transmission, but the overall mosquito population density is reduced by over 40% due to the fitness costs of wMel infection coupled with the impact of CI on wild-type fitness. Overall, the impact of wMel introduction in this scenario is insufficient to reduce the R_0 of dengue to below 1, the threshold for permanent elimination. Nevertheless, *Wolbachia* introduction results in near immediate and transient elimination of dengue transmission for over 10 years post-introduction, with re-establishment of sustained (although substantially reduced) transmission only occurring more than 25 years after the initial release. These dynamics result from wMel introduction disrupting the balance between dengue transmission and host population (i.e. ‘herd’ immunity); wMel introduction reduces the *effective* reproduction number (the reproduction number accounting for human population immunity), R , of dengue to well below 1, causing transmission to cease. Only as herd immunity falls in the human population (due to new immunologically naïve people being born) can transmission resume.

Figure 4 shows how the long-term impact of large-scale wMel establishment on dengue disease varies with the efficiency of maternal transmission of wMel, the local transmission intensity (R_0) of dengue and one further entomological factor which turns out to be important: the intensity of density-dependent regulation of *Aedes aegypti* larval populations. Our default scenario for larval density dependence (used by nearly all mathematical models of mosquito population dynamics) is that the per-capita rate of larval mortality is proportional to larval density. More intense larval density dependent competition (here represented by a mortality rate proportional to the square of larval density) leads to somewhat lower reductions in dengue transmission, consistent with the predicted effects of larval competition on *Wolbachia* establishment discussed earlier [57–59]. As expected, imperfect maternal transmission of *Wolbachia* also reduces impact.

Future challenges

Enormous progress has been made in the last decade to bring us to the current point, with *Wolbachia* positioned as one of the most promising novel interventions to control dengue and other arboviruses. However, several major challenges remain (see Outstanding Questions box). Despite the large impact modelling predicts large-scale *Wolbachia* releases will have on dengue transmission, experimentally evaluating effectiveness in the field is not straightforward [71,72]. Dengue incidence is highly variable over time and over relatively short spatial scales, necessitating either a long duration trial in a small number of locations or a shorter trial in a large number of sites [69,70]. Being an area-based intervention, randomisation in an efficacy trial needs to occur at the site rather than the participant level, necessitating a cluster-randomized design. Furthermore, even in high transmission settings,

annual incidence of clinical dengue rarely exceeds 5% in any age group, requiring large cohorts of trial participants to be recruited to achieve sufficient trial power [71]. Somewhat greater power (or smaller numbers of participants) can be achieved using seroconversion as the trial endpoint, but this necessitates bleeding participants (usually young children) at multiple timepoints over the trial. ‘Contamination’ also presents a challenge; if the *Wolbachia* release areas in a trial are too small, participants resident in those areas may be exposed to dengue when they travel outside those areas, or by wild-type mosquitoes migrating in. Similarly, if control areas are placed close to intervention areas, participants resident in the former may partially benefit from the protection provided by the latter, particularly if *Wolbachia* spreads from the intervention to the control areas over the course of a trial.

These challenges have led to consideration of other more novel study designs to evaluate impact. One promising approach is to adapt the test-negative design used for evaluating influenza vaccine effectiveness [73] to a cluster-randomised trial context. A sample of febrile patients with dengue-like symptoms seeking healthcare are recruited across both intervention and control areas, their residential location determined, and are virologically tested for dengue. If *Wolbachia* reduces dengue transmission, the ratio of test-positive to test-negative patients is expected to be lower in intervention areas than control areas. A cluster-randomized trial of *w*Mel releases using a test-negative end-point design is now underway across the city of Yogyakarta in Indonesia, with results expected in late 2019 (<https://clinicaltrials.gov/ct2/show/NCT03055585>).

An alternative approach to evaluating effectiveness, albeit giving a lower standard of evidence, is simply to release *Wolbachia* across a large urban area and observe the impact upon dengue incidence trends observed through routine surveillance. Given the imperfect specificity of clinical diagnosis of dengue, enhanced virological confirmation of suspected dengue cases is desirable in such studies. While such observational approaches cannot establish causality, should the effect size be as large as the modelling discussed above would suggest, a large reduction in dengue case numbers would be expected. So long as this reduction was sustained, as more observation time accumulated it would be increasingly difficult to attribute the observed reduction to chance effects, particularly if dengue incidence outside the release area continued at comparable levels to previously. Such an approach is being adopted for the two largest scale releases of *w*Mel currently underway – in Medellin, Colombia and Rio de Janeiro, Brazil. By late 2019, sufficient data should have accumulated in both cities to make initial assessments of effectiveness.

Optimistically assuming that the studies currently underway demonstrate that *w*Mel release leads to large falls in dengue incidence, there are additional challenges involved in transforming *Wolbachia* into a fully operationalised, standardised, inexpensive vector control ‘product’, capable of large-scale highly cost-effective deployment. Facilities for the mass-production of mosquitoes have become increasingly sophisticated and resource-efficient over the last few years, due to the development and testing of both *Wolbachia* and genetically modified mosquitoes. However, all *Wolbachia* field releases to date have been very heavily monitored via fine spatial scale grids of mosquito traps, with release sizes and durations being adaptively tuned based on the observed spread of *Wolbachia* into the wild-

type population. Such high levels of instrumentation are less feasible for routine large-scale operational deployment and would add substantially to costs, but more research is needed to optimise standardised release protocols to maximise the chance of successful establishment while minimising the duration of releases and numbers of mosquitoes needing to be released.

As a biological control, we should also expect the phenotypic properties of any one *Wolbachia* strain to potentially evolve over time [74], perhaps leading to reductions in the extent of transmission blocking. In addition, current *Wolbachia* strains introduced into *Aedes aegypti* may not be optimal in their fitness costs, level of blocking or temperature sensitivity [75]. It will therefore be important to maintain development of a pipeline of new strains capable of being successfully released into *Aedes aegypti* populations where *w*Mel has already been established. The encouraging results from experiments examining the degree of viral suppression achieved in *Aedes aegypti* superinfected with both *w*Mel and *w*AlbB [36] suggest that the release of superinfected mosquitoes might be an effective strategy to manage potential reductions in the capacity of single *Wolbachia* strains to block transmission.

Nevertheless, despite these challenges, *Wolbachia* represents a highly innovative and exciting new approach to vector control for arboviral diseases, and the first which offers the real prospect of dramatically reducing the global disease burden caused by those pathogens. Mathematical modelling will continue to have an important role to play in helping to overcome the challenges involved in evaluating the effectiveness of *Wolbachia* and transforming it into a fully operationalised, cost-effective intervention capable of being globally deployed.

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- *Wolbachia* is a vertically transmitted endosymbiotic intracellular bacterium that is naturally present in about 60% of all insect species.
- With the goal of dengue transmission reduction, a number of strains of *Wolbachia* have been deliberately transinfected into *Aedes aegypti*.
- In small-scale field releases, these strains have been shown to spread to high frequencies in *Aedes aegypti* populations.
- *Wolbachia* offers the potential to reduce dengue transmission via two mechanisms: mosquito population density reduction (*Wolbachia* imposes a fitness cost) and reduced vector competence.
- Mathematical modelling has guided our understanding of the spread of *Wolbachia* in mosquito populations, its potential impact on dengue transmission, and field study design.
- City-scale studies are now underway to evaluate the effect of releasing *Wolbachia*-infected *Aedes aegypti* on dengue transmission.
- What is the impact of large-scale releases of *Wolbachia*-infected *Aedes aegypti* on dengue transmission? High spatiotemporal variability in dengue incidence requires studies to be large (geographically and in terms of the enrolled population). Considerable efforts, including mathematical modelling, have been put into study design in recent years. A mixture of cluster randomized trials and observational studies are now underway across multiple cities in Latin America and Southeast Asia.
- What is the best protocol for releasing *Wolbachia*-infected mosquitoes to ensure establishment, minimise cost and reduce the need for high levels of monitoring? Releases to date have adaptively adjusted the numbers of mosquitoes released and the duration of releases to ensure rapid establishment, making use of extensive real-time mosquito trapping data. Transforming *Wolbachia* into an operationalized vector control tool capable of inexpensive and wide deployment will require robust protocols for release which are not reliant on such intensive monitoring.
- What will be the long-term phenotypic stability of the *Wolbachia* strains transinfected into *Aedes aegypti* in the field? As a recently transinfected biological control, it is quite possible that the *Wolbachia* – *Aedes aegypti* system will co-evolve in coming years. Should this result in reduced fitness costs or suppression of arboviral replication, new *Wolbachia* strains may be needed which can superinfect existing populations. As a corollary, learning how *Wolbachia* reduces arbovirus replication in the mosquito may allow higher efficacy strains to be developed.
- If *Wolbachia* releases are insufficient to eliminate dengue in high transmission settings, how can *Wolbachia* best be combined with other control measures, notably vaccines? This is far from obvious, given the one licensed vaccine has

relatively low efficacy in seronegative recipients, and *Wolbachia*, by driving down transmission, will decrease dengue seroprevalence in treated areas.

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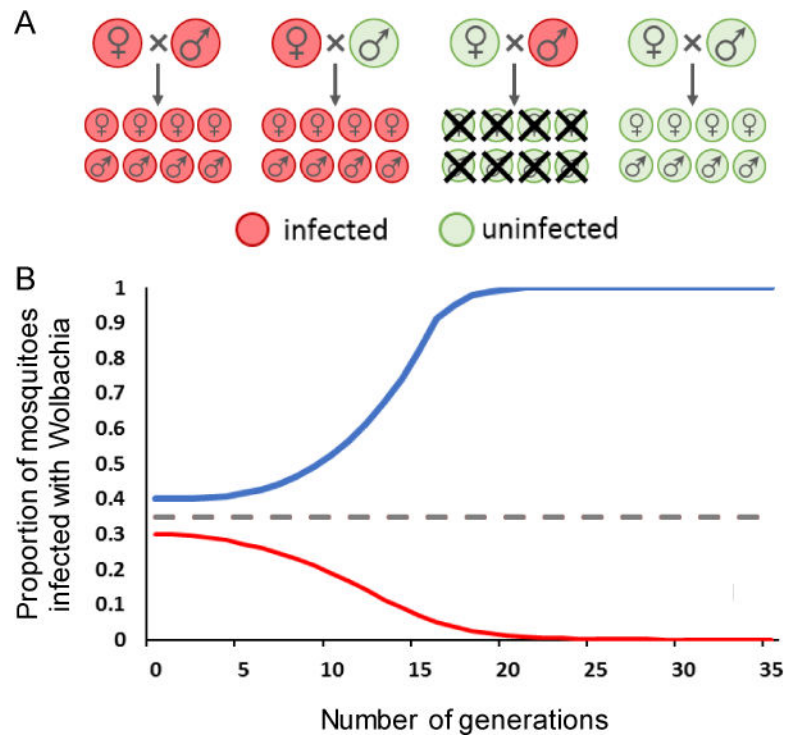


Figure 1. Invasion dynamics of *Wolbachia*

A. Illustration of how cytoplasmic incompatibility (CI) gives *Wolbachia*-infected females a reproductive advantage by making the progeny of infected males and wild-type females non-viable. **B.** CI thus allows *Wolbachia*-infected mosquitoes to displace wild-type (blue curve) if introduced into a population above a threshold frequency (dashed line) which is determined by the fitness costs of *Wolbachia* infection. If introduced below this threshold frequency (red curve), *Wolbachia*-infected insects are out-competed by wild-type, despite CI.

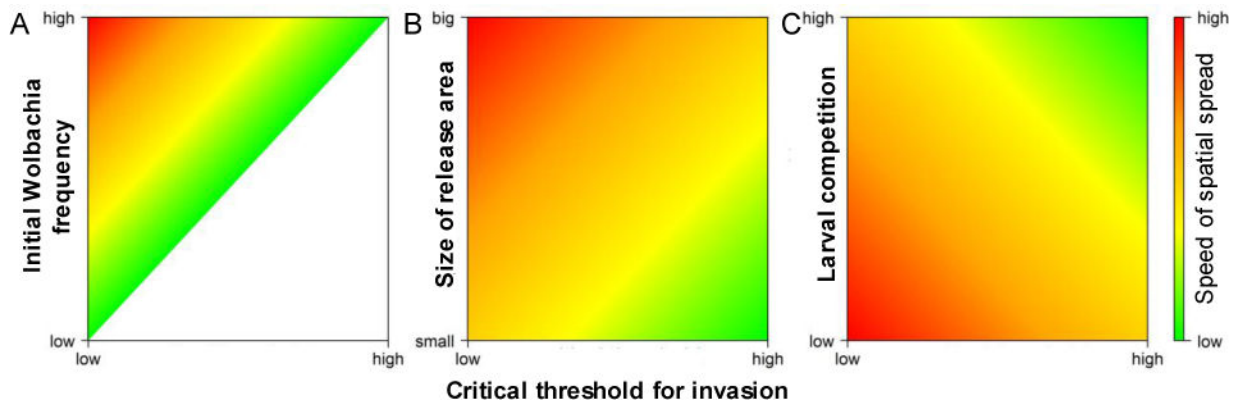


Figure 2. Schematic illustration of factors influencing the spatial spread of *Wolbachia* in a mosquito population

A. In the initial release area, the frequency of *Wolbachia*-infected mosquitoes needs to exceed the critical threshold for invasion (see Figure 1B) for invasion to occur. **B.** *Wolbachia* is more likely to establish and spread spatially if the initial release area is larger. **C.** More intense larval stage density-dependent competition slows initial establishment and spatial spread.

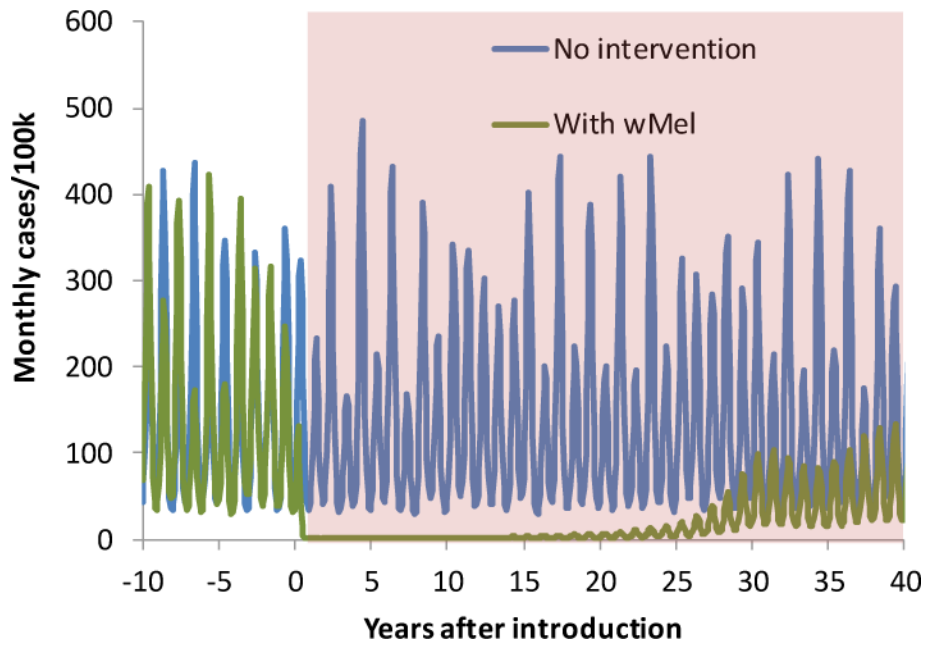


Figure 3. Simulated time-series of monthly symptomatic dengue incidence following (or without) *Wolbachia* release

Results use the deterministic dengue transmission model published in [15]. Mean dengue R_0 set at 4.5 and $wMel$ is assumed to reduce dengue infectiousness of mosquitoes by 40% and to have 90% efficient maternal transmission. Shaded area shows dynamics after the initial release of *Wolbachia* at time 0.

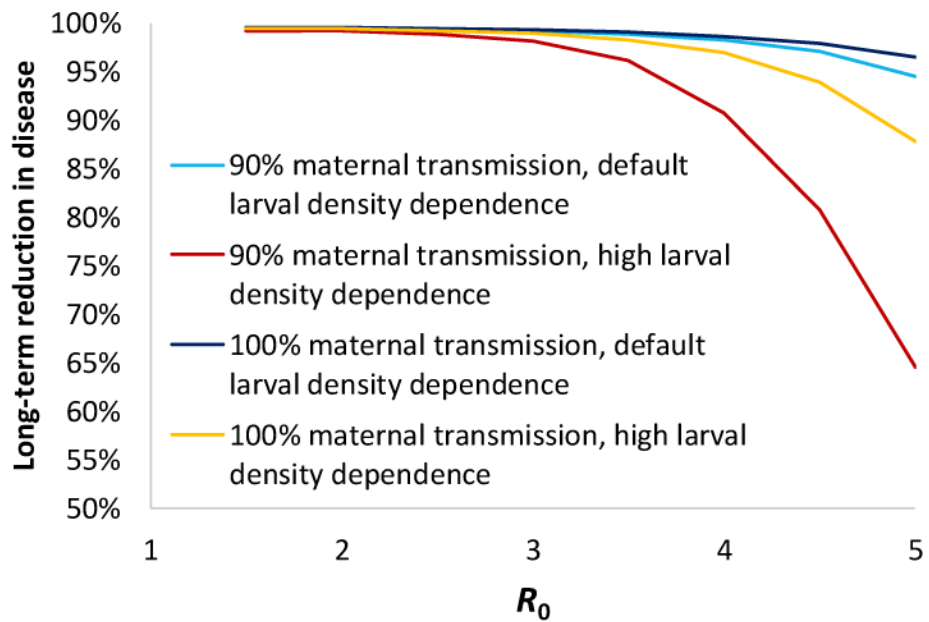


Figure 4. Predicted long-term impact of large-scale *Wolbachia* release on symptomatic dengue incidence derived using the deterministic dengue transmission model published in [15] Four different scenarios using different assumptions regarding *Wolbachia* maternal transmission and larval competition intensity are shown. Note that the transient impact of *Wolbachia* release on dengue transmission (i.e. in the first 10–30 years post-release) is much larger (see Figure 3), typically giving a >95% reduction in dengue cases in that timeframe.