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REVIEWS

Spotlight

Combining Sterile and Incompatible Insect Techniques for *Aedes albopictus* Suppression

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Traditional control strategies are failing to contain *Aedes albopictus* as an emerging major vector for dengue. A combination of approaches (Zheng *et al.*, *Nature*, 2019) involving an artificial triple *Wolbachia* superinfection and low-dose irradiation enabled mass production of adult sterile males for release. The resulting suppression of field populations suggests feasibility for area-wide vector control.

Major mosquito-borne viral diseases such as chikungunya, dengue, and Zika impose a tremendous global health burden, with an increasing prevalence in both tropical and temperate regions [1]. While the *Aedes aegypti* mosquito is the primary vector for the disease-causing arboviruses, the involvement of the closely related *Ades albopictus* species in virus transmission is progressively growing. *A. albopictus* is a vector for dengue, considered one of the world's most prevalent infectious diseases [2,3], as well as chikungunya [4] and Zika [5], which have experienced global expansions with serious impact, as illustrated by the recent Zika pandemic. No drugs or broadly effective vaccines exist for protection against any of these arboviruses; therefore, disease control has largely relied on mosquito elimination and avoidance, with the former being the most common and mostly carried out through chemical insecticides targeting adult mosquitoes. However, insecticide resistance and environmental impact is hampering the control efforts, causing control programs to fail and having a serious public health impact [6,7].

An alternative strategy for mosquito suppression is based on the sterile insect technique (SIT), which relies on the mass release of radiation-sterilized males that will not produce viable offspring after mating with wild-type female mosquitoes. Despite some successes with SIT-based control of agricultural pests, such as screw worms and fruit flies, SIT has not been widely used against mosquitoes because of the fitness impact of irradiation that results in noncompetitive males [8].

Another approach for mosquito population suppression that overcomes the limitations of SIT is the incompatible insect technique (IIT) which relies on the maternally inherited endosymbiotic bacterium *Wolbachia.* Because of the cytoplasmic incompatibility (CI) phenomenon, matings between released *Wolbachia*-infected male mosquitoes and females carrying different types of *Wolbachia* do not produce offspring, but the competitiveness of *Wolbachia*-infected males is only minimally or not compromised. However, the success of this strategy is dependent on the complete removal of *Wolbachia*-infected fertile females during the mass rearing process, since any released infected female would result in the spread of *Wolbachia* in the population. This is because matings between *Wolbachia*-infected females and either infected or noninfected males are compatible and result in *Wolbachia*-infected offspring [8], which would prevent the population suppression achieved through CI and instead result in population replacement (*Wolbachia* noninfected mosquitoes being replaced with *Wolbachia*-infected mosquitoes). Semiautomated mechanical separation of mass-reared male and female pupae can be achieved on the basis of gender-specific size differences, but this technique does not result in the required 100% efficacy.

Zheng *et al.* [8] used a combination of SIT and IIT to successfully overcome this limitation in the deployment of an *A. albopictus* population suppression strategy (Figure 1). Their approach to eliminate the presence of *Wolbachia*-infected females from the mass-reared males was inspired by an early hypothesis that a combination of low-dose irradiation and pupal size-based separation can remove all fertile females from a *Wolbachia*-infected male pool without severely affecting the males' fitness or mating performance.

Another complication with the application of IIT with the *A. albopictus* species is its natural superinfection with the two native *w*AlbA and *w*AlbB *Wolbachia* strains. Zheng *et al.* [8] used embryo microinjection to transfer a *Culex pipiens*-associated *Wolbachia* strain *w*Pip into the natively superinfected *A. albopictus* wild-type line (Figure 1). This triple *Wolbachia*-infected mosquito HC strain produced complete cytoplasmic incompatibility-mediated sterility when HC males were crossed with non-*w*Pip infected females, and *w*Pip could be stably maintained via 100% maternal transmission through successive generations. The presence of wPip only minimally compromised the mating competitiveness of the HC males when compared with their wild-type counterparts. An additional benefit of acquiring *w*Pip infection was a significantly reduced ability of the HC females to transmit both dengue and Zika viruses. The combined application of efficient sex separation through mechanical size selection and low-dose irradiation with the introduction of *w*Pip into the *A. albopictus* wild-type line met the required conditions for IIT control programs.

After laboratory and field-cage experiments in conjunction with modeling, optimization of mosquito mass-rearing, and approval from the Chinese Ministry of Agriculture,

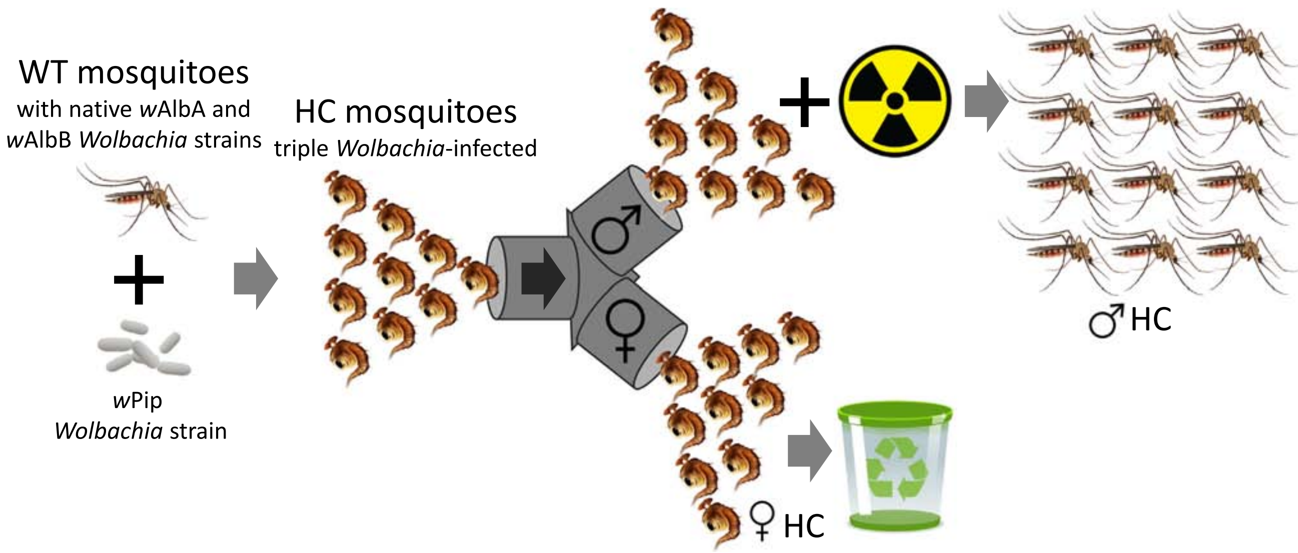

Figure 1. Simplified Illustration of the Sterile Insect Technique (SIT) and the Incompatible Insect Technique (IIT) Combination Strategy for Mosquito Control based on triple infected HC female mosquitoes. The approach involves the introduction of a *Culex pipiens*-associated *w*Pip *Wolbachia* strain into the *Aedes albopictus* wild-type (WT) line with two native *w*AlbA and *w*AlbB *Wolbachia* strains, to produce the triple infected HC line, and the efficient sex separation through mechanical size selection and low-dose irradiation.

Dr Xi's team initiated open-release field trials in 2014 in the Guangzhou province of the People's Republic of China, where *A. albopictus* is the only (and highly abundant) vector that is responsible for the country's most intense dengue transmission. The first pilot-scale releases, albeit involving smaller numbers of mosquitoes because of mechanical and labor-intensive manual sex separation (rather than low-dose irradiation), resulted in 62% and 65% yearly reductions, respectively, in mosquito population at two selected field sites. Application of pupal irradiation, rather than manual sex separation, in 2016 and 2017 allowed for the production and release of much larger numbers of mosquitoes and resulted in 83% and 94% yearly mosquito reductions, respectively. These reductions were highest in relatively isolated areas, whereas areas containing transportation routes appeared to be resistant to this mosquito-control campaign because of human activities that facilitated mosquito introduction into release areas. The negligible number of recaptured *w*Pip-positive female mosquitoes and larvae in the release areas indicated that the sex separation was indeed highly effective and that population replacement was not occurring. The competitive mating ability of released HC males that had been treated with low-dose radiation was only 50–70% of that of the wild-type males, but this negative effect was offset by the large number of males released. The community support for the HC *A. albopictus*-based population suppression control program dramatically increased from 13% to 54% as a result of the significantly reduced mosquito prevalence achieved, which resulted in a 96.6% and 88.7% decreased biting rate in the two release areas [8].

The study by Zheng *et al*. [8] showed a near-complete elimination of two *A. albopictus* field populations through the combined use of IIT and SIT with *w*Pip-infected *A. albopictus*. The *w*Pip-mediated refractoriness to the viruses added an additional layer of safety against the resumption of disease transmission, even in the case of unintended population replacement as a result of imperfect sex separation and thus the release of *w*Pip-infected females. Nevertheless, population suppression carries some additional benefits for disease control, such as a reduction in biting nuisance and a greater public acceptance. Population suppression also protects against the introduction of possible emerging mosquito-borne pathogens that would not be sensitive to a *Wolbachia*-mediated refractory mechanism. The efficacy of this control approach was highly dependent on the ease and likelihood of mosquito migration into the release areas, thus making it more suitable in more isolated urban habitats and islands. The application of combined IIT- and SIT-based mosquito control in larger and less isolated areas would require improvements in mass rearing, sex separation, and release to allow for the use of much larger numbers of sterile male mosquitoes.

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Forum

The Uncertainty Surrounding the Burden of Post-acute Consequences of Dengue Infection

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Post-acute consequences currently form a significant component of the dengue disability-adjusted life year (DALY) burden estimates. However, there is considerable uncertainty regarding the incidence, duration, and severity of these symptoms. Further research is needed to more accurately estimate the health and economic burden of these dengue manifestations.

The Burden of Dengue

Dengue is the most prevalent mosquito-borne viral disease affecting humans in the world today, occurring mainly in the tropics and subtropics. Symptomatic infection commonly presents as a mild to moderate acute febrile illness lasting 2–7 days[i] [1]. Headache, nausea, vomiting, muscle and joint pains, and rash, are prominent features. Although most infected individuals recover from the acute episode without complications, a small proportion of patients can develop potentially life-threatening disease during the critical phase known as severe dengue[i]. A key metric to measure and compare the burden of different diseases is the disability-adjusted life year (DALY): one DALY is equivalent to one healthy life year lost. A key reason why DALYs are used to quantify disease burden is that this metric incorporates the burden of nonfatal health outcomes, as focusing only on mortality gives an incomplete picture of the actual burden of a disease[ii]. An overview of the approach used to estimate DALYs for dengue is presented in Box 1.

The Global Burden of Disease (GBD) 2013 and 2017 study estimates for dengue are summarized in Figure 1[iii] [2]. When breaking down the DALY burden by its different components, it is apparent that, despite only applying to 8.5% of symptomatic cases, the estimated healthy life lost due to post-acute chronic symptoms (Box 1) contributed notably to the burden estimate (Figure 1). This demonstrates that the post-acute consequences/sequalae of dengue are a key driver of the overall DALY burden estimates. This article aims to explore the variation in the definitions and incidence of these post-acute consequences, and how these may influence the uncertainty around estimates of dengue's health and economic burden.

The Post-acute Consequences of Dengue Infection

A range of post-acute consequences of dengue have been reported across different studies [3]. The most consistently reported included myalgia, weakness, headache, fever, and fatigue [3–7]. However, some of these symptoms, such as fever or headache, are quite common and are prevalent during an acute dengue episode. Defining the end of an acute dengue episode can be difficult, and it is unclear if any of these symptoms were truly carried on into the post-acute period or misclassified as post-acute. Other symptoms also reported include vomiting, diarrhoea, nausea, chills, and poor appetite [4]. Both hospitalized patients and outpatients can experience these post-acute consequences and they have not been shown to associate with the severity of the acute episode [4,8]. Furthermore, post-acute consequences were usually associated with female gender and older age [3,4,9].

The estimated proportion of patients experiencing post-acute consequences varies significantly across studies and tends to decrease over time after the acute illness [3,6]. Figure 2 illustrates the percentage of patients reporting dengue symptoms that may result in productivity losses (fatigue, asthenia, or trouble working) by elapsed follow-up time. However, there were differences in how the various studies defined and quantified these symptoms. For instance, Seet *et al*. [4] used a validated fatigue questionnaire to assess particular symptoms relating to the physical and mental status of their patients. In contrast, other studies have only quantified the number