Chapter 11

Use and release of mosquitoes for the control of dengue transmission: A world-first trial in Australia

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Mosquito-borne diseases such as malaria or dengue fever cause a huge health burden to people living in tropical and subtropical countries. Current control efforts are not always effective and many of these diseases have increased in prevalence, geographic distribution and severity. The transinfection of Aedes aegypti *mosquitoes with the endosymbiotic bacterium* Wolbachia pipientis *is a promising biocontrol approach for those diseases. Naturally occurring* Wolbachia *strains have been stably introduced from fruit flies into mosquitoes and shown that these strains can invade and sustain themselves in mosquito populations while blocking the replication of dengue viruses and other pathogens inside the insects. This chapter discusses the release of* Wolbachia*-infected* A. aegypti *mosquitoes in North Queensland, Australia. The regulatory process for this kind of release had no precedent in Australia and was authorised after a thorough community engagement process and an independent risk assessment. At the time of writing (April 2012), a second release trial was currently underway in Queensland and the technology will soon be deployed in dengue-endemic areas of Southeast Asia and in Brazil, once appropriate approvals are in place.*

Introduction

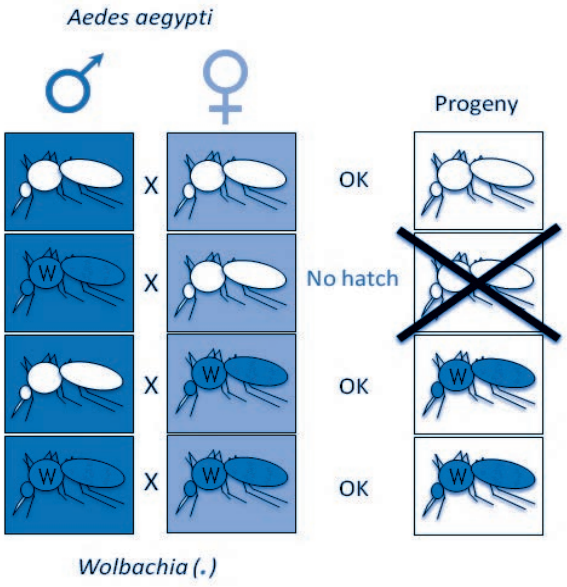
Mosquito-borne diseases are one of the major threats to human health. The malaria parasite transmitted by anopheline mosquitoes in particular causes an enormous health burden mainly among African children, and kills about 1 million people every year (World Health Organization, 2008). The second most deadly mosquito-borne disease, dengue fever, is caused by an RNA virus transmitted primarily by the bite of female *Aedes aegypti* (yellow fever mosquitoes). Causing about 50 000 deaths every year and affecting between 50-100 million people, this disease has increased in severity and distribution, and is now affecting more than 100 countries in tropical and subtropical regions of the world (Kyle and Harris, 2008; World Health Organization, 2009). *A. aegypti* mosquitoes are highly anthropophilic and breed in water containers around houses (old tyres, vases, fallen palm tree fronds, discarded items, etc.), therefore rapid urbanisation in developing countries has contributed to increasing mosquito populations and the concomitant spread of dengue. There are currently no effective vaccines or specific treatments for dengue fever nor the most severe form of the disease dengue haemorrhagic fever (Wilder-Smith et al., 2010), therefore disease monitoring and mosquito control programmes are the only preventive methods currently available. Traditional control approaches for dengue have targeted the mosquito by spraying insecticides, reducing breeding sites or using predatory copepods and fish to eliminate larvae (Kay and Vu, 2005), but these approaches can be very costly and they have not proven as effective as desired, in particular due to the rise of insecticide resistance (Kyle and Harris, 2008; Morrison et al., 2008). More recently, there has been a clear increase in activities related to the development and release of genetically modified (GM) mosquitoes, particularly to control the dengue and malarial vectors. The first generation of transgenic mosquitoes designed to suppress *A. aegypti* populations by effectively using a method similar to the sterile insect technique were released in the Cayman Islands in November 2009 (Reeves et al., 2012), while another release took place in Pahang, in Malaysia, between 2009 and 2012. These releases have been somewhat controversial and have not always been preceded by publication of the associated hazards and their regulatory approval processes (reviewed by Reeves et al., 2012).

The use of *Wolbachia* as a biocontrol agent

A new biocontrol strategy that does not involve genetic modification and does not have the environmental risks associated with the use of insecticides is currently being developed for the control of dengue. This approach uses *Wolbachia pipientis*, an intracellular alpha-Proteobacterium that is a very common endosymbiont of insects and other arthropods, but does not infect vertebrates and is harmless to humans. It is estimated that up to 76% of all insect species harbour *Wolbachia* infections, making this probably the most prevalent microbial symbiont in the biosphere (Hilgenboecker et al., 2008; Jeyaprakash and Hoy, 2000). These bacteria, discovered in the 1920s in the ovaries of *Culex* mosquitoes (Hertig and Wolbach, 1924), frequently induce a series of reproductive distortions in their insect hosts (Werren et al., 2008), the most common being cytoplasmic incompatibility (CI), a form of embryonic lethality that occurs when *Wolbachia*-infected males mate with uninfected females (Figure 11.1). The CI gives *Wolbachia*-infected females a reproductive advantage over uninfected ones, allowing *Wolbachia* to spread into populations (Hoffmann and Turelli, 1997), since these bacteria are maternally (vertically) transmitted through the egg cytoplasm. *Wolbachia*'s invasion ability has tremendous potential for the control of mosquito-borne diseases as they could be used to

spread antiparasitic traits into insect populations, with the intention of making them refractory to disease. Alternatively, *Wolbachia*'s CI phenotypes could be used to render mosquito populations incompatible and induce population suppression. The use of *Wolbachia* for the control of mosquitoes was postulated as early as the 1960s (Laven, 1967), and some preliminary field trials were done temporarily in Burma and India to control *Culex* mosquitoes (Curtis and Adak, 1974).

Figure 11.1. **Schematic representation of the cytoplasmic incompatibility phenotype induced by *Wolbachia* in *Aedes aegypti* mosquitoes**

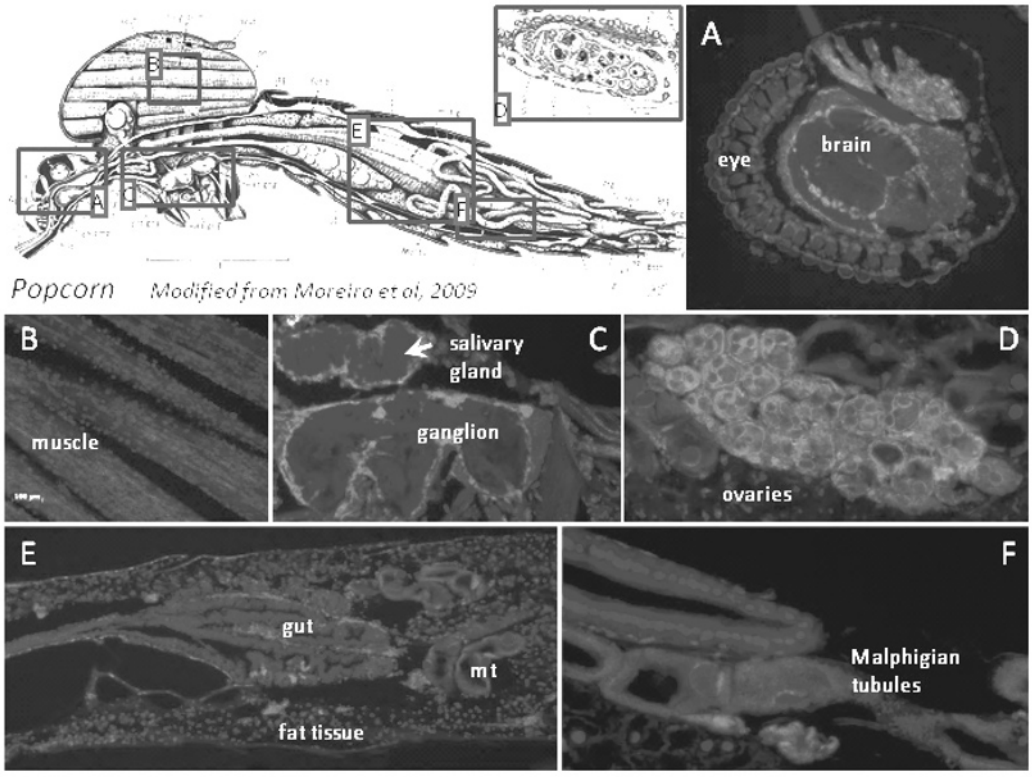


Out of the hundreds of different *Wolbachia* strains present in insects, a strain named *popcorn* (*w*MelPop) appeared to be particularly promising for the control of mosquito-borne disease. This strain, originally discovered in *Drosophila melanogaster* fruit flies in 1997 (Min and Benzer, 1997) over-replicates to high densities in fly tissues and induces CI in infected hosts, while reducing lifespan by about 50%. This is important because the longevity of insect vectors is a key factor affecting disease transmission. Insect-transmitted pathogens, such as dengue viruses or malaria parasites, require a period of replication within the mosquito body before they can be transmitted to another person bitten by the vector. This time, termed the extrinsic incubation period, usually takes about two weeks, a large proportion of the insect's lifespan. Therefore, only the older insects in a population are capable of transmitting dengue (Salazar et al., 2007). The idea behind the use of *Wolbachia* for dengue biocontrol was relatively simple; *popcorn Wolbachia* could be stably introduced into *A. aegypti* mosquitoes, which contain no *Wolbachia* infections in the wild, and CI would allow the bacterial infection to spread within the mosquito population, while eliminating the older (disease transmitting) individuals (Sinkins and O'Neill, 2000; McMeniman et al., 2009).

Despite *Wolbachia* being extremely common symbionts of insects and other arthropods, including some mosquito species, *A. aegypti* mosquitoes are not naturally infected with this bacterium. Therefore, for this approach to work, the *Wolbachia* infection must be transferred to mosquitoes in the laboratory using technically challenging methods such as embryonic microinjection. In 2006, two stably transinfected mosquito lines containing *popcorn Wolbachia* were generated following thousands of

embryo injections (McMeniman et al., 2009). Initial efforts using *Wolbachia* isolated from *popcorn*-infected *D. melanogaster* flies were unsuccessful. Infected mosquitoes were finally obtained after using *Wolbachia* that had been maintained in *A. albopictus* cell lines *in vitro* for several years with continuous serial passage (McMeniman et al., 2008). It is believed that this period of adaptation to a similar host intracellular environment was a key factor for the success of the microinjection, and cell adaptation approaches are being used for the generation of additional infections in other mosquito species. *Popcorn*-infected *A. aegypti* mosquitoes contain very high *Wolbachia* densities and they are widely distributed in most tissues including fat bodies, muscle, nervous tissue, salivary glands, Malphigian tubules, and in particular, ovaries (Figure 11.2) (Moreira et al., 2009). Strong ovarian infection is important for the stability of the transinfected lines, as it allows the bacteria to spread to the female progeny at extremely high rates and be maintained in the population once the initial infection has been created.

Figure 11.2. **Fluorescence *in situ* hybridisation of paraffin sections**



Note: This figure shows the localisation of *Wolbachia* (in red) in different tissues of *A. aegypti*. 8 µm sections were hybridised with two *Wolbachia* specific probes labelled with rhodamine (Moreira et al., 2009). DNA is stained with DAPI (blue). The top diagram has been adapted from Jobling (1987). (A) Head section showing *popcorn Wolbachia* in the brain and ommatidia. (B) *Wolbachia* in the thoracic muscle. (C) Salivary gland and thoracic ganglion. (D) Ovaries. (E) Midgut, fat tissue and Malphigian tubules (mt). (F) Malpighian tubules.

The presence of *popcorn Wolbachia* in mosquitoes reduces their adult lifespan by about 50% (McMeniman et al., 2009; Yeap et al., 2011), similar to the original infected fly hosts (Min and Benzer, 1997). *Wolbachia* also induce strong CI in *A. aegypti*, which allows the infection frequency to increase in the population. However, the most interesting effect from the *popcorn* infection in *A. aegypti* was discovered in 2009, when

Moreira et al. (2009) found that the bacteria have a strong inhibitory effect on dengue virus replication within the mosquito body. *Wolbachia*-infected mosquitoes have dramatically reduced dengue levels compared to uninfected counterparts after being fed on dengue-infected blood or being injected in the thorax with dengue viruses. These decreased dengue titers were confirmed by RT-PCR and also in immunostaining studies that showed the absence of dengue in the presence of *Wolbachia* (Moreira et al., 2009). Numerous recent studies have found similar inhibitory effects against a variety of insect-borne pathogens and insect viruses, including the Chikungunya virus, *Plasmodium*, *Drosophila* C virus, cricket paralysis virus, filarial nematodes, West Nile virus, etc. (Moreira et al., 2009; Panteleev et al., 2007; Hedges et al., 2008; Teixeira et al., 2008; Osborne et al., 2009; Kambris et al., 2010; Bian et al., 2010; Glaser and Meola, 2010; Hughes, G.L. et al. 2011). The molecular basis for the interference between *Wolbachia* and dengue remains unknown, although the two main hypotheses to explain it are based on the upregulation and priming of the mosquito immune system by the novel *Wolbachia* infection (Moreira et al., 2009; Kambris et al., 2009; Rances et al., 2012), and the direct competition for resources between *Wolbachia* and dengue viruses (Moreira et al., 2009; Iturbe-Ormaetxe et al., 2011).

A second *Wolbachia* strain (*w*Mel) from *D. melanogaster* flies was introduced into *A. aegypti* in 2009 by embryo injection (Walker et al., 2011). This strain is very closely related to *popcorn*, and is globally distributed in wild *Drosophila* populations (Riegler et al., 2005) and does not significant induce life-shortening in their native fly host or in transinfected *A. aegypti* (Walker et al., 2011). *w*Mel induces complete CI in mosquitoes and is also less abundant in *Aedes* tissues and as a result has lower fitness costs to the mosquitoes than *popcorn*, and as such, has stronger potential to spread into uninfected populations (Yeap et al., 2011; Turelli, 2010). Interestingly, *w*Mel also blocks DENV replication, although at slightly lower levels than *popcorn* (Walker et al., 2011), which makes it a very good candidate for a release trial. The potential of *w*Mel to spread and invade insect populations is further demonstrated by the global invasion of this strain in *D. melanogaster* during the past 80 years (Riegler et al., 2005), where it replaced a strain more closely related to *popcorn*.

Field releases of *Wolbachia*-mosquitoes in Australia: The regulatory process

The Eliminate Dengue Program[1] is a multinational project primarily funded by the Foundation for the National Institutes of Health through the Bill and Melinda Gates Grand Challenges in Global Health Initiative, and is aimed at using *Wolbachia*-infected *A. aegypti* as a novel strategy for the control of dengue. This programme is led by Australian scientists but includes international collaborators from Brazil, Indonesia, the People's Republic of China, Thailand, the United States and Viet Nam.

Subsequent to the encouraging scientific data, and in preparation for a pilot release of *Wolbachia*-infected mosquitoes in Australia, contained semi-field cages were constructed at James Cook University in Cairns, north Queensland, Australia (Ritchie et al., 2011). The environment in these greenhouse-like cages mimicked the typical Cairns backyard garden and contained potted plants surrounded by mulch, as well as a structure simulating the understory of a traditional north Queensland home, a classic spot where *A. aegypti* usually rest in this area. Cohorts of *Wolbachia*-infected mosquitoes were released into a wild-type population and the experiments demonstrated that both *w*Mel and *popcorn*-infected *A. aegypti* were able to invade and successfully replace uninfected

populations of mosquitoes, reaching fixation in the cages within one to three months (Walker et al., 2011).

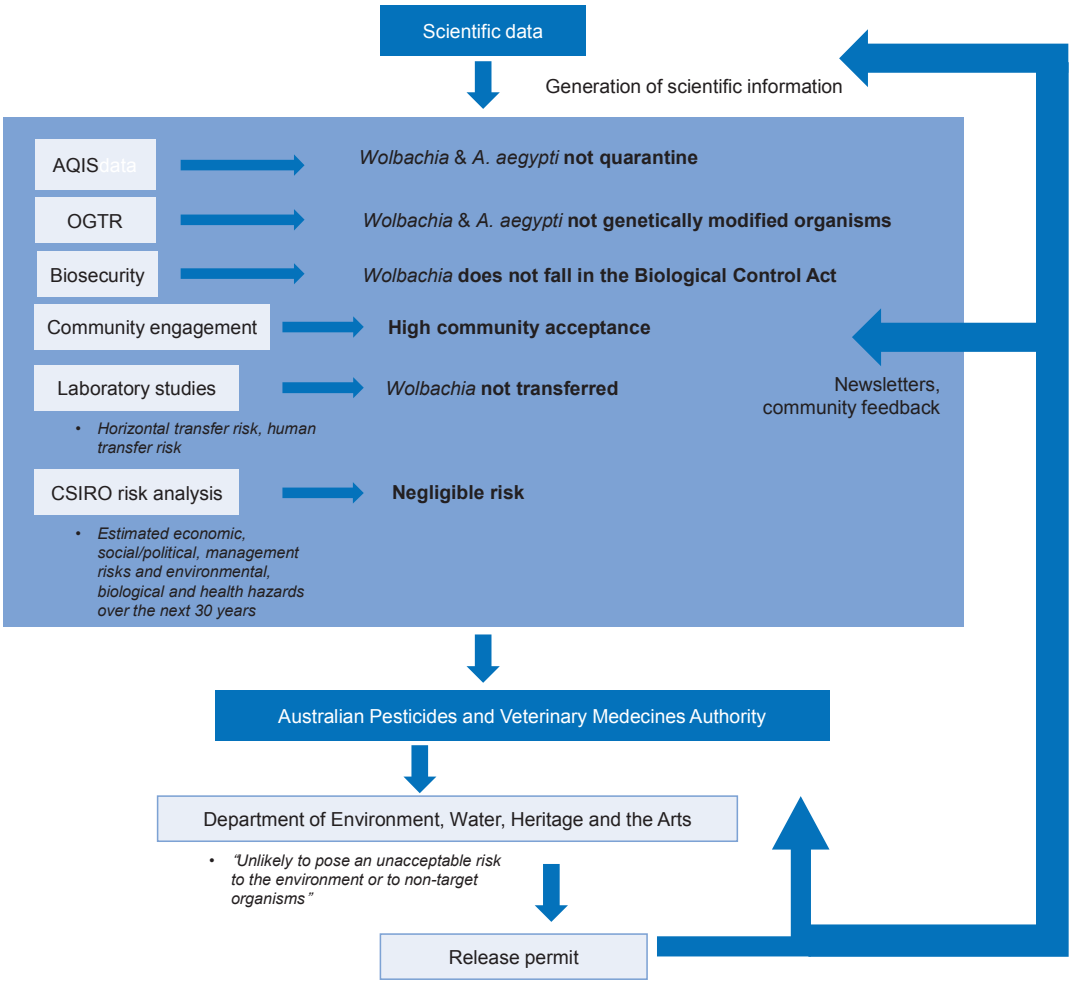
Following the promising results from the laboratory and field-cage studies, a research trial involving the open release of mosquitoes into dengue-prone areas of northern Queensland, Australia was planned. The release of *Wolbachia*-infected mosquitoes for biocontrol purposes had no precedent in Australia, therefore the regulatory pathway for this trial had to be mapped out. Australia has a very strict approach to the importation and release of exotic organisms into the environment and there are four major pieces of legislation that regulate it: the Quarantine Act 1908, the Biological Control Act 1984, the Environment Protection and Biodiversity Conservation Act 1999 and the Gene Technology Act 2000 (De Barro et al., 2011).

Figure 11.3 illustrates the process that took place before the release permit was granted. After initial consultation, the Australian Quarantine and Inspection Service (AQIS, now DAFF) ruled out that *Wolbachia* are not subject to quarantine as they naturally occur into the Australian environment, and as such are not regulated under the Quarantine Act. In fact, studies have revealed that *Wolbachia* are quite prevalent in Australian insects and arthropods, including some iconic species that are common in the release areas, such as the Cairns birdwing butterfly, or very well-known arthropods such as huntsman spiders or fruit flies.[2] Humans have constantly been exposed to *Wolbachia*-infected insects, either by sharing their environment, being bitten by them or by consuming plant products that are infected or contain residues from these insects – even by directly eating *Wolbachia*-infected insects as part of some diets or culinary traditions. Moreover, as up to 76% of all insect species are naturally infected with *Wolbachia* (Hilgenboecker et al., 2008; Jeyaprakash and Hoy, 2000), probably many of the insects deliberately released into the environment for other biocontrol purposes have been inadvertently infected with these bacteria.

Following the assessment by AQIS, the Chief Biosecurity Officer in Queensland determined that *Wolbachia* was not a foreign biological organism, and as such did not fall within the Biological Control Act. Similarly, the Office of the Gene Technology Regulator (OGTR) in Australia, who decides on licence applications to release genetically modified organisms, concluded that *Wolbachia*-infected mosquitoes were not within its remit, because neither the mosquito nor the bacteria have been genetically modified and they can be considered a biological control agent, but not a GMO. In fact, no genetic transformation technologies have yet been developed for *Wolbachia* despite extensive attempts by various laboratories, so all biocontrol efforts are focused on using the traits found in wild type strains. The fact that neither organism in the *Wolbachia-Aedes* association is genetically modified has been a key contributing factor to the relatively fast deployment of this strategy in the field, given the current public and regulatory hurdles to the release of genetically modified organisms in Australia and many other countries.

Regulatory approval for the release was finally granted by the Australian Pesticides and Veterinary Medicines Authority (APVMA), which decided to regulate *Wolbachia* as a "veterinary chemical product" (Figure 12.3). This was based on § 5(2) of the Agriculture and Veterinary Act 1994, that defines a veterinary chemical product as "a substance that is used for application to an animal by any means, as a way of directly or indirectly modifying the physiology of the animal so as to alter its natural development or reproductive capacity" (De Barro et al., 2011).

Figure 11.3. **Regulatory pathway followed in Australia for the release of *Wolbachia*-infected *Aedes aegypti* mosquitoes for the control of dengue**



Note: The release permit granted by the APVMA requires the generation of reports on the spread of *Wolbachia*. The affected communities are informed about the results. These releases have generated a large amount of scientific data that will facilitate further releases.

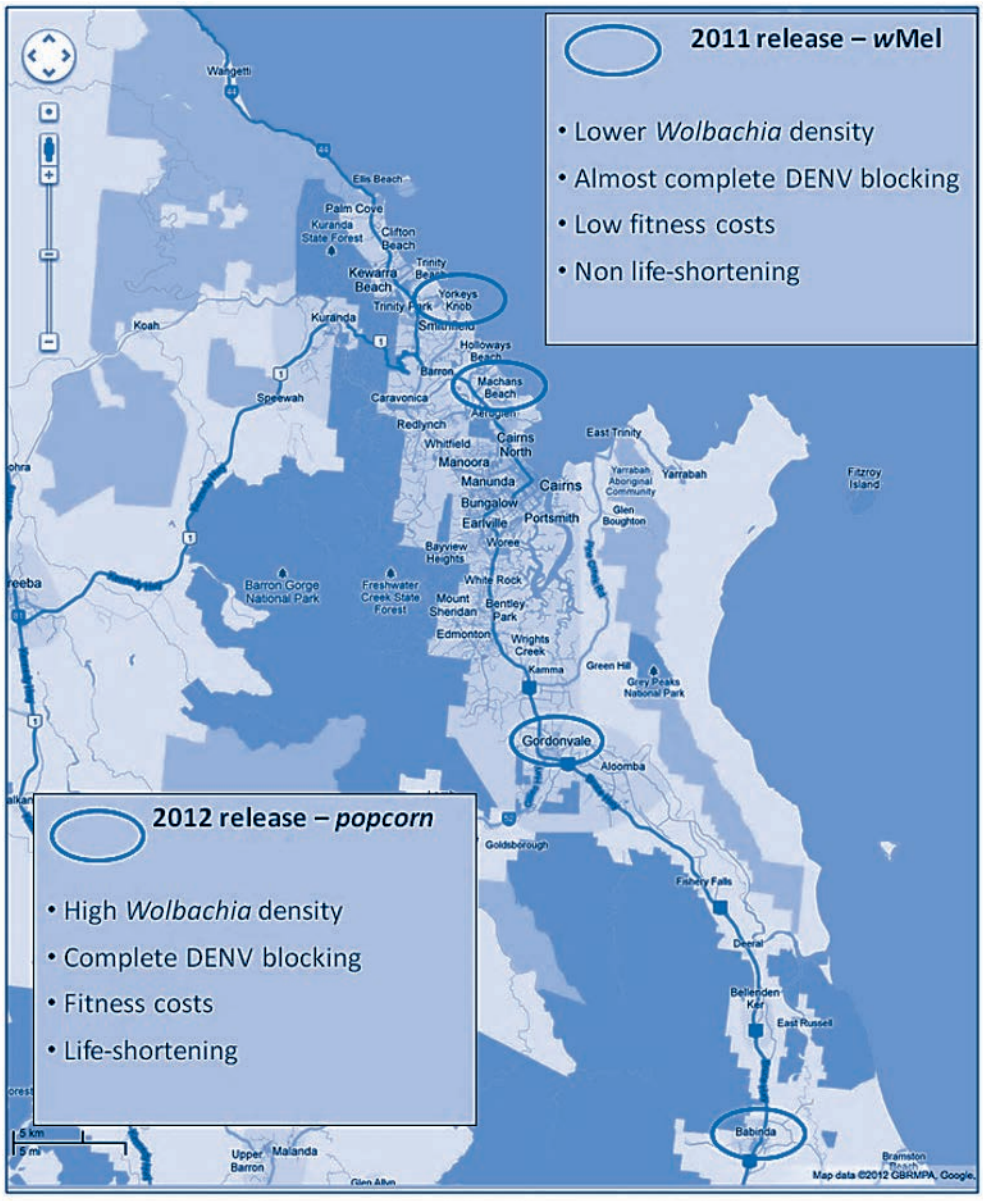
Key for the approval of the release by the APVMA was the risk analysis study conducted by the Commonwealth Scientific and Industrial Research Organisation (*CSIRO*). During an eight-month period, an independent panel of experts estimated the economic, socio-political, management, environmental, biological and health hazards over the next 30 years, determining the likelihood and consequences of these. Fifty hazards were initially considered and later grouped into 30 main hazards (Murphy et al., 2010), which included harm to the environment, the local economy, the tourism industry, human health, even the risks of people perceiving that if this strategy was successful there was no further need to be vigilant against mosquitoes. This study concluded that there was a "negligible risk (lowest possible rating) that the release of *Wolbachia-A. aegypti* will result in more harm than currently caused by naturally occurring *A. aegypti* mosquitoes over a 30-year period".

The APVMA also undertook a further risk assessment with the support from the Federal Commonwealth's Government Department of Sustainability, Environment, Water, Population and Communities, which supported the release. As part of the environmental risk assessment by the APVMA and the CSIRO, as well as community concerns identified during the social studies that took place in the release sites before release, laboratory studies were conducted to demonstrate that *Wolbachia* is not transmitted to humans during mosquito biting (Popovici et al., 2010). The sera from human volunteers that have blood fed thousands of *Wolbachia*-mosquitoes during the course of the project was compared to sera from control individuals that never fed these mosquitoes, and no evidence of *Wolbachia* antibodies in the sera of blood feeders was found. This is likely due to the fact that *Wolbachia* bacteria are too large (0.5-1µm) to pass through the mosquito salivary duct during feeding. These studies also showed that *Wolbachia* are not stably transferred to non-target species that feed on mosquito larvae (spiders, fish or crustacean predators) or share the environment where the mosquitoes live, and they cannot survive in the environment (plants, soil) where mosquitoes are kept (Popovici et al., 2010). Despite the fact that *Wolbachia* are extremely common in many arthropod species, natural horizontal transfer events are extremely rare, and the wide distribution of *Wolbachia* among insects is explained by the many millions of years that *Wolbachia* is believed to be associated with insects.

Wolbachia establishment in north Queensland mosquito populations

Between January and April 2011, up to 300 000 *A. aegypti* mosquitoes infected with the *w*Mel *Wolbachia* strain were released in the localities of Gordonvale and Yorkeys Knob, near Cairns, north Queensland (Figure 11.4) (Hoffmann et al., 2011). Adult (male and female) mosquitoes bred at the Mosquito Research Facility at James Cook University were placed in plastic cups and released weekly on ten occasions at every fourth house. The release was preceded by the removal of water from breeding containers in these sites one month earlier, to reduce the local *A. aegypti* population and maximise the proportion of *w*Mel mosquitoes. Only households that agreed on the release were targeted. The thorough community engagement process and the information campaign that preceded the release, together with the desire of people to participate in a novel dengue control strategy, generated extremely high community support. In order to monitor the spread and invasion of *Wolbachia*-infected mosquitoes in the release sites, a grid of up to 320 mosquito ovitraps were deployed in houses within and around the release areas. Collected eggs were hatched, reared into 2nd-3rd instar larvae, and then sent to a molecular lab in order to test for the presence of *Wolbachia*, as well as to determine whether the larvae were *A. aegypti* or not, by PCR. These studies demonstrated that the *Wolbachia* infection was able to spread and invade the release areas within four months, with percentages of *Wolbachia*-infected mosquitoes rising from 0% to above 80-90% in Gordonvale and Yorkeys Knob just before the dry season (Figure 11.5) (Hoffmann et al., 2011). These percentages reached 100% when the mosquito population was tested again at the beginning of the next wet season (unpublished data), showing that the *Wolbachia* infection has become fixed in these sites. None of the thousands of non-*A. aegypti* eggs collected during this period in the traps and tested by PCR were found to be infected with *w*Mel *Wolbachia*, which highlights the lack of horizontal transfer among mosquito species co-habiting in the same environment.

Figure 11.4. **Location of the 2011 and 2012 *Aedes aegypti* release sites in north Queensland, Australia**

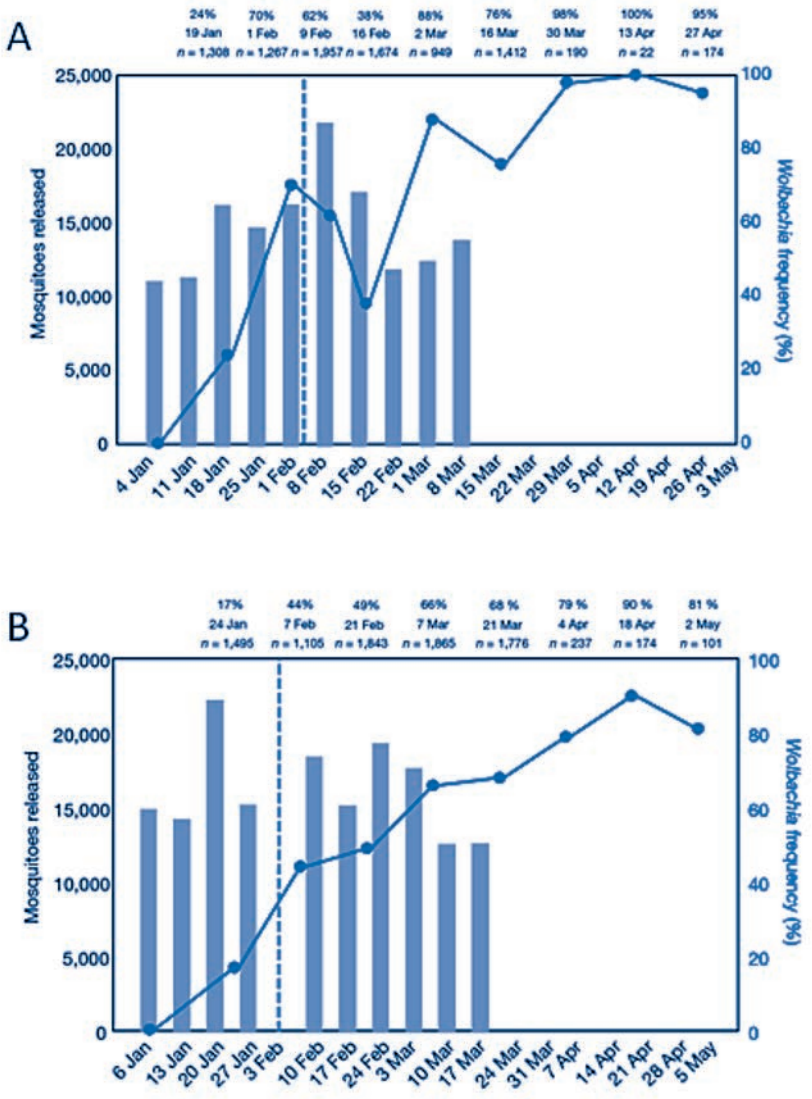


Note: The main phenotypes induced by the *w*Mel and *popcorn Wolbachia* strains in transinfected mosquitoes are described. This document and any map included herein are without prejudice to the status of or sovereignty over any territory, to the delimitation of international frontiers and boundaries and to the name of any territory, city or area.

During the 2012 wet season (January-April), a second release trial took place in the localities of Machans Beach and Babinda, near Cairns, following further support from the local communities. This release was supported by an amended permit from the APVMA, based on the submission of reports from the first release. This time, *A. aegypti* mosquitoes infected with the *popcorn* strain were used. This *Wolbachia* strain, although conferring more fitness costs to the mosquitoes, has much stronger dengue-blocking abilities than *w*Mel, and as such might represent a better alternative in dengue-endemic countries. Of particular interest will be to determine whether these mosquitoes are able to spread and

then survive the dry season, since the presence of *popcorn Wolbachia* has been shown to affect female fecundity and the survival of desiccated eggs (McMeniman and O'Neill, 2010). So far, the *popcorn* infection has spread in Machans Beach and Babinda, and at the time this chapter was written in April 2012, almost 80% of the *A. aegypti* mosquitoes in these areas were infected with this strain.

Figure 11.5. **Increase in the frequency of *Wolbachia*-infected mosquitoes in Gordonvale and Yorkeys Knob during the 2011 release**



Notes: In grey (bar graph), the number of mosquitoes released; in green (line graph), *Wolbachia* frequency. The dotted line indicates the time when tropical storm Yasi landed near Cairns, disrupting some of the monitoring collections.

Source: Hughes, G.L., et al. (2011), "*Wolbachia* infections are virulent and inhibit the human malaria parasite *Plasmodium falciparum* in *Anopheles gambiae*", *PLoS Pathogens*, No. 7, e1002043.

In order to minimise the spread of *Wolbachia*-infected mosquitoes to non-target areas during the trials, only release sites that were isolated from neighbouring localities by physical barriers to *Aedes* dispersal (highways, sugar cane fields, forests, the ocean) were chosen (Hemme et al., 2010). A key safety consideration addressed by the APVMA is the monitoring of *Wolbachia* in neighbouring areas, therefore a grid of ovitraps was also deployed in various localities adjacent to the release sites (Hoffmann et al., 2011). Only small numbers of *Wolbachia*-infected *A. aegypti* were detected occasionally in some areas near the release sites, probably due to movement through vehicles or adult dispersal. Modelling studies have shown that the proportion of *Wolbachia*-infected mosquitoes must be above a threshold before a successful invasion takes place, so even if a small number of mosquitoes were to be dispersed to new sites, they would find it very difficult to establish a persistent local infection and would be easily swamped by wild-type mosquitoes (Barton and Turelli, 2011). Currently, there is no evidence to suggest that *w*Mel has been able to establish in neighbouring areas.

Future directions for *Wolbachia*

This novel strategy for dengue control has clearly demonstrated that, at least in the Australian environment, *Wolbachia*-infected mosquitoes can successfully invade and replace native uninfected populations when released in sufficient numbers. The establishment of *Wolbachia*-infected mosquitoes in the field should facilitate the future deployment of this strategy to other countries. Additional releases would no longer require the labourious rearing of thousands of adult mosquitoes in the laboratory but could instead be implemented by relocating field-collected mosquito eggs from infected sites to naive locations.

Determining whether these mosquitoes will have an actual effect on dengue transmission cannot be easily resolved in Australia, since dengue is not endemic in the country and the number of cases can vary enormously from year to year, depending on reintroductions from infected travellers (Gould and Solomon, 2008). Such a large epidemiological study is only feasible in dengue-endemic areas and this is now being proposed for countries such as Brazil, Indonesia or Viet Nam, where future deployments of *Wolbachia*-infected mosquitoes are currently being prepared. The Australian trial is being used as a template to develop community engagement strategies and risk assessment analyses for these settings, as well as for paving the pathway for regulatory approval in these countries.

Wolbachia-based strategies are well advanced in *A. aegypti,* where other strains have also been introduced, such as the *w*AlbB *Wolbachia* strain from *A. albopictus* (Xi et al., 2005), but they are not limited to this mosquito species (Iturbe-Ormaetxe et al., 2011). *A. albopictus*, an invasive species that has spread from Asia to the United States, Africa and southern Europe (Gratz, 2004) and is a secondary vector for dengue and Chikungunya, was very recently stably transinfected with *w*Mel *Wolbachia*, which also induces CI and blocks dengue transmission in this species (Blagrove et al., 2012). *A. albopictus* are dengue vectors despite being naturally infected with two *Wolbachia* strains, *w*AlbA and *w*AlbB (Sinkins et al., 1995). Other mosquitoes, such as *Armigeres subaltatus* or *A. fluviatilis*, are also naturally infected with *Wolbachia* strains, and are vectors for Japanese encephalitis virus (Tsai et al., 2006) and *Plasmodium gallinaceum* (Moreira et al., 2009), respectively. The work by Blagrove et al. and previous studies (Hedges et al., 2008; Osborne et al., 2009) have shown that not all

Wolbachia strains have the same pathogen interference phenotypes, and choosing the right genotype is essential for the approach to work.

Alternative technological strategies for disease control

The use of *Wolbachia* symbionts for the control of mosquito-borne disease is compatible with the use of alternative strategies currently being developed, such as vaccines, as well as traditional approaches such as the use of insecticides. *Wolbachia* mosquitoes add to the arsenal of disease control weapons being considered, such as the development of genetically modified mosquitoes expressing anti-parasitic molecules or the creation of paratransgenic approaches that uses symbiotic or gut-associated recombinant bacteria that express this molecules (reviewed by Caragata and Walker [2012], and see Chapter 12). The main scientific challenge with these approaches are the identification of pathogen or mosquito targets that can be engineered to reduce disease, as well as the development of mechanisms that allow the maintenance and spread of these genes in the populations. Obtaining the regulatory and the community consent to release these organisms into the environment may be the more difficult hurdle to overcome. The emphasis from the Eliminate Dengue team on communication with the local community before, during and after the releases was crucial for the acceptance and success of the strategy.

Although the release of *Wolbachia* mosquitoes in Australia was obviously not regulated as a genetically modified organism, the social, scientific and risk studies that preceded it, together with the success of the deployment strategy, can serve as a very interesting model of regulation of mosquito releases. The Australian regulatory experience also revealed that despite the approach being beyond the regulatory process for GMOs, the level of scrutiny with regards to biosafety was very rigorous (De Barro et al., 2011). This strategy is planned to be further tested in the future, when additional releases are carried out in South East Asian countries.

A comprehensive list of *Wolbachia* literature and resources can be found at the *Wolbachia* website[3] and full information about the field release of *Wolbachia*-infected mosquitoes for dengue control is also available online.[4]

Notes

1. www.eliminatedengue.com.
2. www.eliminatedengue.com.
3. www.wolbachiawebsite.org/index.html.
4. www.eliminatedengue.com.

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