Releasing mosquitoes to control mosquitoes

Geneticist Luke Alphey and his team describe how they use genetically modified mosquitoes to reduce the number of mosquitoes that spread dengue fever

Key words

Virus
Disease
Dengue fever
Gene cloning
Recombinant DNA technology

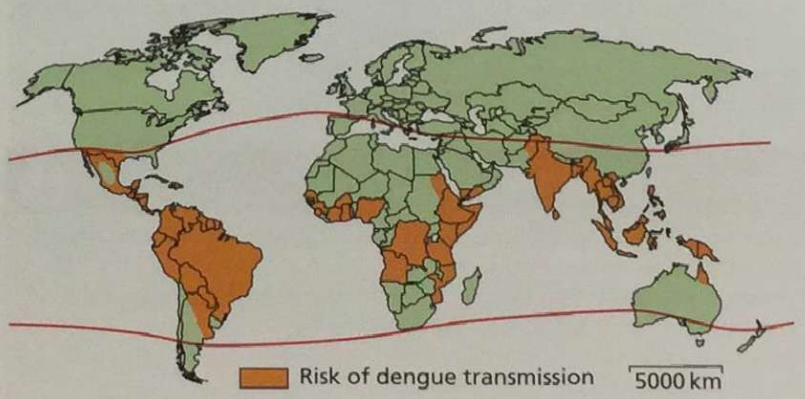
Dengue fever is the fastest growing mosquito-borne disease — 50–100 million people are affected each year. It is a viral disease spread by female mosquitoes, and largely by females of just one species — *Aedes aegypti*. This mosquito takes blood mainly from humans (see Figure 1), and tends to live in and around people's homes. Dengue can result in fever, headaches, tiredness and muscle and joint ache. In its more severe form — dengue haemorrhagic fever — the disease can be fatal. Four different but related viruses cause this disease. If a person becomes infected by one strain of virus, they can develop immunity to that strain, but then they become more susceptible to the three other strains. Infection with a second viral strain usually produces more severe symptoms.

In recent years the human population at risk from dengue fever has increased to over 2.5 billion (40% of the world's population). This disease is found in most tropical areas, although the majority of cases are in Asia, Latin America, the Caribbean and the Pacific (see Figure 2). However, the mosquito that transmits dengue is spreading rapidly, and it is now found in over half the states in the USA. There is no known cure or treatment for dengue fever. Currently, the only way to try to control the disease is by controlling the number of mosquitoes, typically by using pesticides.

Figure 1 Female *Aedes aegypti* feeding on human blood

Using mosquitoes to reduce mosquito numbers

An exciting new way of reducing the number of mosquitoes that transmit the dengue virus has recently been developed (see Figure 3). This method uses genetically modified (GM) mosquitoes to control mosquitoes, and takes advantage of the fact that male mosquitoes do not bite humans and so do not transmit the disease. How this works is explained in more detail in the next section, but basically GM mosquitoes that carry a conditionally lethal gene have been made. This means that the gene is usually lethal to the insects, but its activity can be suppressed in the laboratory using a dietary



Data from the World Health Organization

Figure 2 Map showing countries at risk of dengue fever in 2008

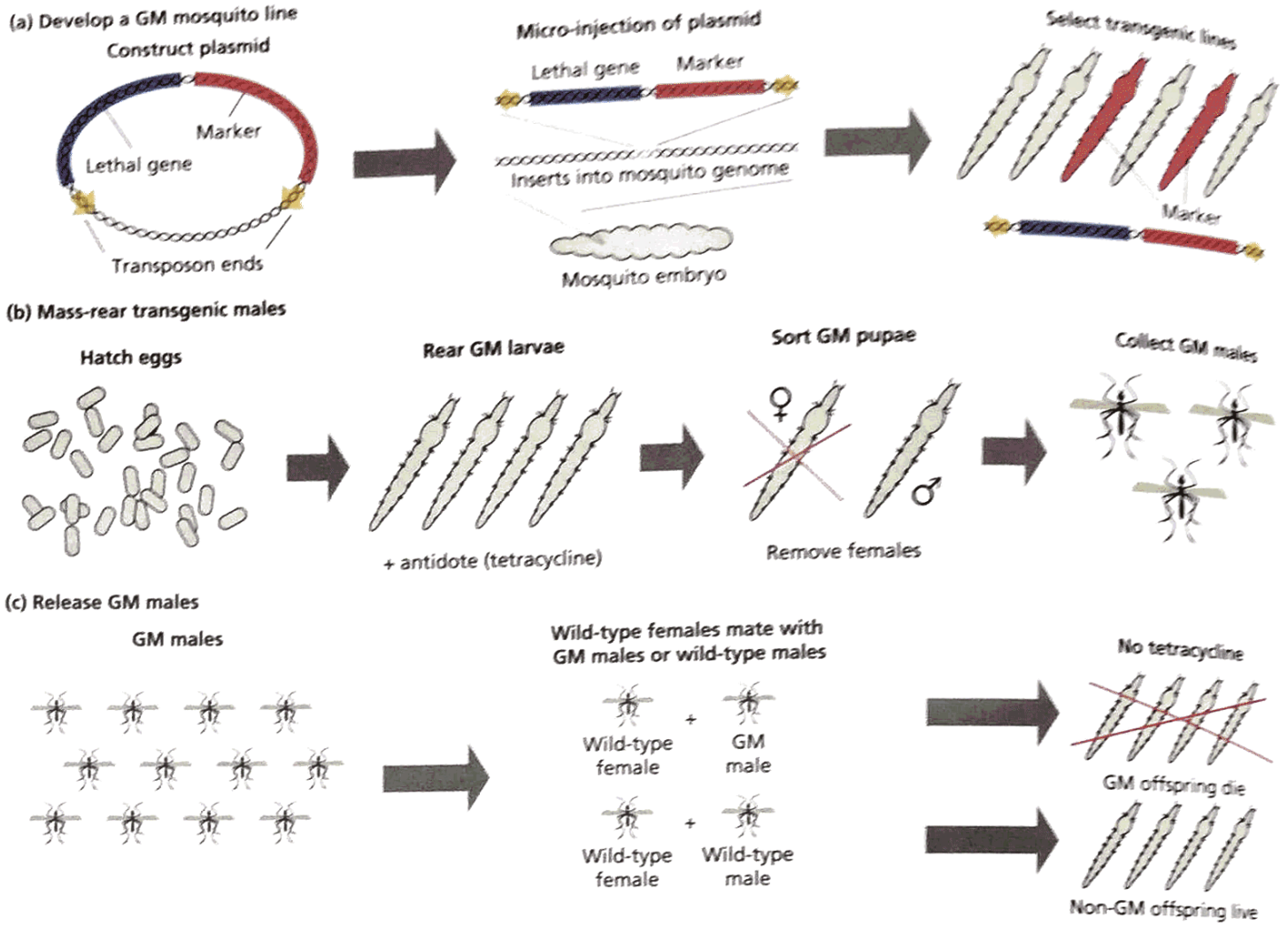


Figure 3 Schematic to show how GM mosquitoes are made and how they work in the environment. Note that the red marker shown in (a) can only been seen using a fluorescence microscope in the laboratory. Neither GM mosquitoes nor their larvae appear red to the naked eye

supplement. This gene is only lethal to developing larvae — it doesn't kill adult mosquitoes. So if adult males that carry this gene — GM males — are released into the wild, they will seek out and mate with wild-type females. Any offspring produced will inherit the lethal gene and will not survive to adulthood. With repeated releases of enough GM males, the mosquito population is reduced.

How the GM mosquito is made

Using genetic modification (see Box 1) a piece of DNA is inserted into the *Aedes aegypti* mosquito genome. This DNA contains:

1 A gene that is active — in this case, lethal — only under certain conditions (**conditional lethal**). In the presence of a specific dietary supplement (tetracycline), the gene that causes lethality is switched off, but in the absence of this dietary supplement the gene is turned on. The product of the gene is a protein called tTA. In the absence of the supplement, tTA builds up to high concentrations in the developing mosquito larvae, stopping them from expressing their normal repertoire of genes. Cell function is disrupted, resulting in cell death, which ultimately kills the developing larvae. Larvae are particularly affected by the build-up of tTA, as this is the stage in which the developing insects need to go through a precise programme of gene expression. Adult mosquitoes are not usually affected by high levels of tTA because they undergo little cell division or development. This means that the adult GM males will survive in the wild, and will find and mate with females. Any offspring produced will contain the lethal gene; tTA will be expressed and the immature mosquitoes will die.

2 A fluorescent marker. The genetically modified insects express a gene encoding a fluorescent protein. This means that insects containing the gene can be identified in the laboratory using a fluorescence microscope (see Figure 4). It also allows the insects to be tracked in the wild. By sampling developing larvae and counting the ratios of

Terms explained

Conditional lethal A lethal trait, or the gene which causes that trait, which is only active under certain conditions.

Epidemic An outbreak of disease in which the number of new infections substantially exceeds what is expected based on recent experience.

Plasmid A small circular DNA molecule that is commonly found in bacteria and can replicate independently of chromosomal DNA.

Transgenic An organism that has been genetically modified by the addition of genes from another species.

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fluorescent to non-fluorescent insects, we can work out how far the **transgenic** insects have travelled from the release site, and how successful they have been at mating with wild-type females.

Sounds easy?

To generate one successful strain of GM mosquitoes (a population of mosquitoes which have the same genetic insertion, see Box 1), many eggs must be injected (see Figure 5). Only a small proportion of these eggs incorporate the inserted DNA into germ line cells and thereby give rise to transgenic offspring. In a typical example, a skilled scientist injected 1500 eggs. Two hundred of these survived the injection process and produced adults. From the many thousands of offspring produced, four new transgenic individuals were found.

Box 1 How are the genetically modified mosquitoes made?

1 A DNA sequence that contains the required gene is inserted into a small loop of DNA known as a **plasmid**.

2 The plasmid also contains some DNA from a transposable element. Transposable elements are regions of DNA that can transpose ('jump') from one DNA molecule to another. In this case, the aim is that the transposable element, along with the genes encoding the conditional lethality trait and the fluorescent marker, will 'jump' from the plasmid into the mosquito's DNA. This step requires an enzyme called a transposase, which cuts out the transposon from the plasmid and reinserts it elsewhere in the mosquito's genome.

3 Plasmids containing the transposable element and the required genetic traits are injected into freshly laid mosquito eggs, along with another 'helper' plasmid which encodes the transposase. Inside the cells of the developing mosquito, the transposase enzyme allows the transposable element, along with the conditional lethality and fluorescent genes, to 'jump' from the plasmid into the mosquito genome.

4 All eggs that survive microinjection are reared in the presence of the dietary supplement and the adults are bred with non-modified mosquitoes. If the DNA in the injected mosquitoes is integrated into cells that go on to produce eggs or sperm — germline cells — some of the offspring produced will inherit the new DNA as part of their genome. These genetically modified offspring are referred to as transgenic because they carry genetic material from another species. Since one of the genes on the injected DNA encodes a fluorescent protein, this acts as a marker, which allows the rare transgenic offspring to be distinguished from the much larger number of non-transgenic siblings.

5 The individual transgenic mosquitoes are mated with wild-type mosquitoes. The offspring from this cross that carry the transgene can again be identified by the presence of the fluorescent marker. Further breeding allows the number of GM mosquitoes to increase and a colony of transgenic mosquitoes can be established. This is called a new transgenic strain.

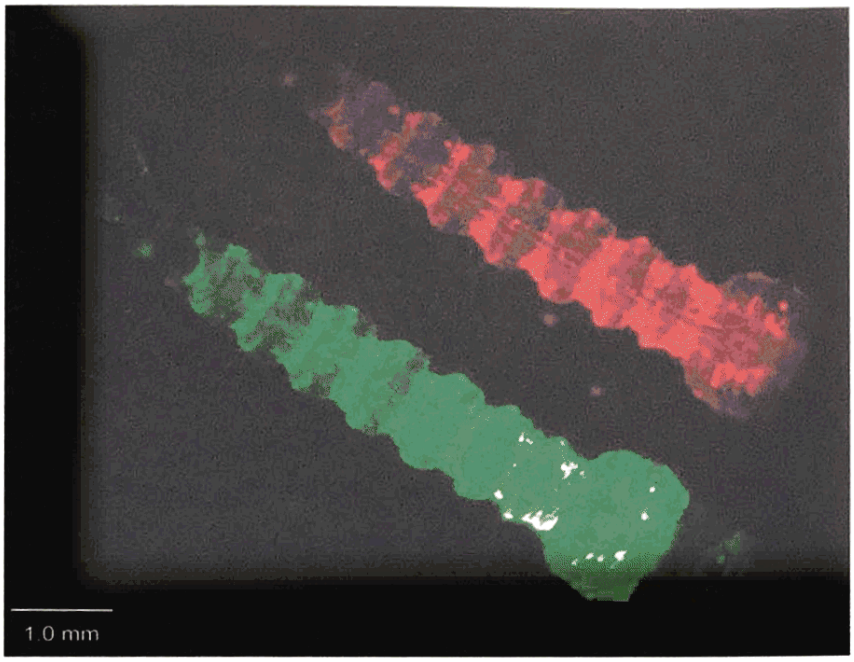

Figure 4 Two transgenic *Aedes aegypti* larvae from different strains expressing different fluorescent markers (red, above; green, below)

The newly identified transgenic mosquitoes are each bred with wild-type mosquitoes as separate lineages (lines). Each transgenic line is slightly different, because there are many sites in the mosquito genome into which the injected DNA can insert. The insertion site can affect the expression of the transgene, due to interactions with the surrounding genes. Therefore, each of the different lines has to be assessed to see which has the 'best' features. Lines that have a high death rate when not given the dietary supplement and a good survival on the dietary supplement are chosen. This is because they will be easy to rear in the laboratory but their offspring will die in the wild. It can take a year or more to develop one GM line, so they are carefully maintained and reared in special laboratories.

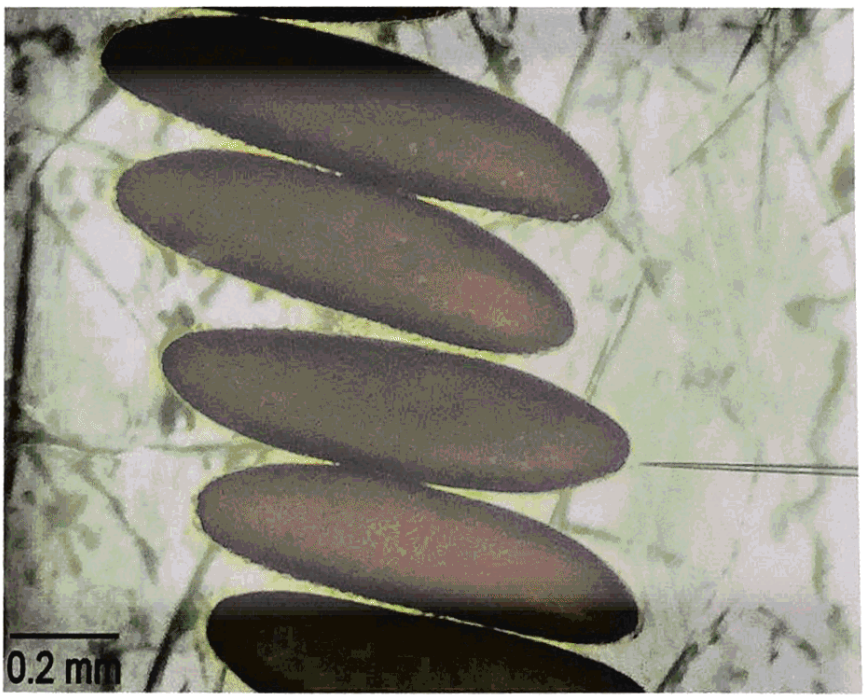

Figure 5 Mosquito eggs and the needle used to microinject them

Does it work?

One of the GM mosquito lines has been used in open field trials in countries including the Cayman Islands and Brazil. Such trials require the permission of the relevant national regulatory authority, which in turn involves rigorous analysis of any potential risks to human or environmental health. Good communication with the people at the trial site is essential as the normal public health message is that mosquitoes are bad.

Release is simple — male GM mosquitoes are released from cages at set intervals in the chosen site. These males disperse into the surrounding area to find and mate with wild females. Compared with insecticide use, where all breeding sites or all houses require treatment, this is an efficient method. The impact on the population can be seen within a couple of months. Suppression of mosquito numbers can be maintained by continuing to release more GM males. Fewer GM males are needed in this 'maintenance' phase than in the initial 'suppression' phase because there are fewer wild males to compete with them for mating with wild females.

Results from the Grand Cayman trial showed that the mosquito population was reduced by 80% in a few months (see Box 2). Even better results were obtained in a trial in Brazil, where the mosquito population was reduced by 96%. This was probably because the Brazilian site was better isolated, with fewer wild mosquitoes flying in from outside the treatment area. Importantly, computer models suggest that 80% suppression would be enough to prevent **epidemic** dengue in most places where it occurs, while 96% suppression would prevent epidemic dengue essentially anywhere.

This new method of controlling mosquitoes has several advantages over pesticides:

- It is highly species-specific: the released male GM mosquitoes will only mate with the females of their own species. This means that no other insect is affected.
- It harnesses the female-seeking behaviour of the male mosquitoes, so unlike a pesticide the GM males will actively seek out the females.
- Pesticides that could have harmful environmental effects are no longer required.

These results are exciting because they could mean that the incidence of dengue fever could be drastically reduced using this new technology without harming the environment.

Further reading

A video describing how GM mosquitoes can be used to fight disease: **http://tinyurl.com/k4wueux**
A video explaining a field trial in Grand Cayman: **www.youtube.com/watch?v=_nY_AlWe5kM**
A video explaining how the technology works: **www.oxitec.com/oxitec-video**
Further information is provided at: **http://tinyurl.com/n8om37b**
World Health Organization factsheet on dengue fever: **www.who.int/mediacentre/factsheets/fs117/en/#content**
The dengue website of the US Centers for Disease Control: **www.cdc.gov/dengue**
Singapore is an example of a country that works hard to control dengue: **www.dengue.gov.sg**

Box 2 Results from the Cayman Island field trial

A GM mosquito line (see Figure 3) has been used in a field trial in the Cayman Islands.

The town was divided into three areas (see Figure 2.1): A (red) — the treatment site where GM mosquitoes were released; B (yellow) — the buffer zone, which helped to make sure that GM mosquitoes were unlikely to fly to the non-treated site, which could have affected the results; C (green) — the non-treated control site.

The mosquito population was measured using an ovitrap — a device that is attractive as an egg-laying site for female mosquitoes.

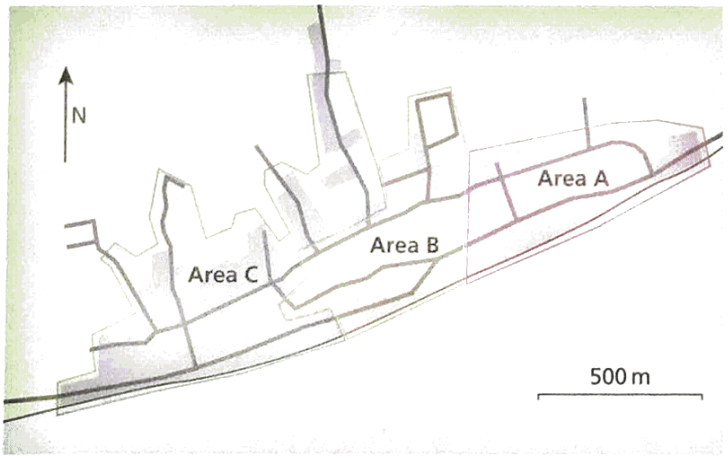

Figure 2.1

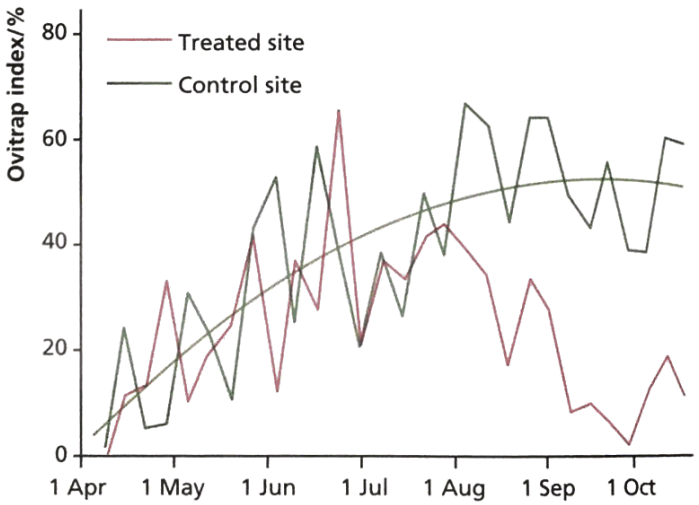

Figure 2.2

The ovitrap index is the percentage of ovitraps containing eggs after a week in the field. Figure 2.2 shows that the mosquito population increased at the start of the rainy season in both areas (April–July). But once females mated with GM males and had started laying eggs, fewer mosquitoes were found in the treated site (A) compared with the control site (C). By October, the number of mosquitoes in the treated area had decreased to about 20% of that in the control region.

Points for discussion

- If you lived in a tropical country where dengue was a significant problem, would you consider using this approach to control it? Do you know anyone who does live in such a country? If so, what do they think?
- What do you think are the advantages and disadvantages of the approach described in this article compared to other methods for controlling dengue?
- To what other diseases or insect pests do you think such a method might be applied?
- Do you think there are any ethical problems with using this type of genetic technology for controlling dengue? What if it were used against an agricultural pest? What if such technology could be applied to fish, or mice?
- What similarities or differences do you see, particularly in terms of ethical considerations, with other uses of genetic technology such as in crop plants, vaccines or insulin production?

Dr Luke Alphey is a visiting professor at Oxford University and is the chief scientist at Oxitec Ltd. **Michael Conway** is a DPhil student and **Esther Miller** is a regulatory specialist. Oxitec successfully conducted the world's first trial using a GM insect in the USA in 2006, and in 2010 showed that a wild mosquito population could be reduced using this approach. Dr Alphey and Oxitec have won a number of awards for this pioneering green technology.