Minireview
Natural and engineered mosquito immunity
Luke Alphey*†

Addresses: *Oxitec Ltd, Milton Park, Oxford, OX14 4RS, UK. †Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 2PS, UK. Email: luke.alphey@oxitec.com

Abstract

A recent paper in *BMC Microbiology* shows how suppression of mosquito innate immunity against a virus that the mosquito can normally tolerate increases mosquito mortality. This is just one of several approaches that may soon bring genetics-based mosquito control methods from the laboratory into the field.

Mosquitoes transmit some of the most deadly infectious diseases of humans. Although malaria is the best known, mosquitoes also transmit a wide variety of viruses and other pathogens. Arthropod-transmitted viruses (arboviruses) include the causative agents of dengue, yellow fever, West Nile virus, chikungunya, and many others. The life cycle of these viruses typically depends on transmission from a suitable vertebrate host via a mosquito vector to another suitable vertebrate, and so on for ever. For some of these viruses, such as dengue, humans are the only suitable vertebrate species across most or all of their range; others, such as West Nile virus, can infect a wide range of vertebrates. The mosquito is exposed to the pathogen when she (only female mosquitoes bite) takes a blood meal from an infectious vertebrate. The virus infects the mosquito, typically first in the midgut and then disseminating through the body. When the salivary glands become infected, so that virus is present in the mosquito's saliva, she becomes infectious. The next time she takes a blood meal, her food source is exposed to the virus. If this individual becomes infected, for a period of time it will become infectious to other mosquitoes that bite it, and so the virus continues to propagate and spread.

Although insects lack the adaptive immune system of mammals, they are by no means merely passive hosts and vectors for these viruses; rather, they have multiple innate immune defenses against the various microbial challenges they encounter. RNA interference (RNAi) is one of the mosquito's major defenses against arboviruses, and suppression of this pathway has previously been shown to increase viral load in infected mosquitoes [1,2]. Two recent papers shed more light on the role of this system in insect antiviral innate immunity. Writing in *BMC Microbiology*, Cirimotich *et al*. [3] show that Sindbis virus engineered to express a suppressor of RNAi produces much more virus than normal in infected mosquitoes, and that this engineered virus is lethal to a range of mosquito species. Previous studies used transient knockdown of components of the RNAi pathway; Cirimotich *et al*. use a protein that binds to double-stranded RNA (dsRNA) and presumably protects it from processing in the RNAi pathway. Although either approach might have pleiotropic effects, both indicate a key role for the RNAi pathway in reducing virus replication and titer. In this regard, in a recent paper in *Nature*, Saleh *et al*. [4] show that *Drosophila* can mount a systemic RNAi-based response to viruses so that uninfected cells at distal locations can prepare a defense against infection. This response was shown to depend on a dsRNA uptake pathway; mutant flies defective in this pathway are hypersensitive to infection with *Drosophila* C virus and Sindbis virus.
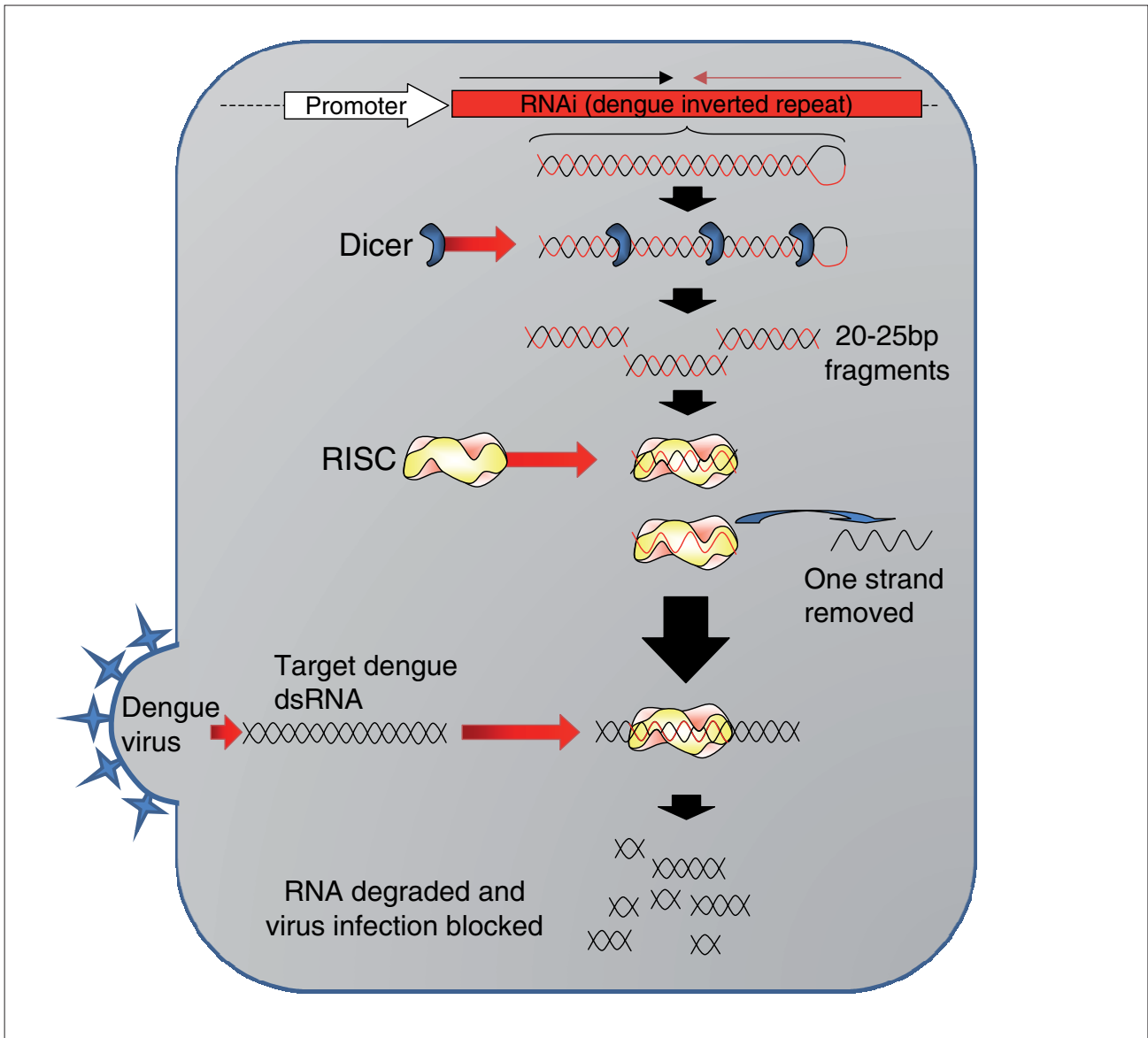
Figure 1
Targeted RNA interference against dengue virus infection
Self-complementary RNA with sequences from dengue virus is expressed from a promoter that expresses in the gut of the mosquito soon after a blood meal [5]. This RNA folds into a hairpin conformation with an extended double-stranded region. This double-stranded RNA is cut into 20-25bp fragments by Dicer. These fragments are bound by the RISC complex of proteins and one strand is removed. The RISC complex is now primed to bind and cleave target sequences from an infecting dengue virus, preventing translation from the RNA and replication of the virus.

Boosting mosquito immunity

As well as their interest in terms of basic immunology, the mosquito's antiviral defenses are significant from an applied perspective. If they could be artificially boosted to the point that infected mosquitoes do not themselves become infectious, mosquitoes that cannot transmit a specific virus, or perhaps even a range of viruses, could be produced.

Antiviral RNAi has already been used to confer resistance to dengue virus in transgenic mosquitoes, by expressing a hairpin RNA corresponding to part of the virus [5]. This long hairpin has the significant advantage of being relatively resistant to mutation of the virus target, as it presumably targets multiple viral sequences. Constitutive expression of a large hairpin RNA may be deleterious, but this potential

problem was minimized by using a promoter that expresses only in the midgut - the first cells to be infected - and only following a blood meal.

Spreading a new immunity gene through a wild population

A virus-resistant strain of mosquitoes in the laboratory is, however, only a curiosity or a research tool. To have an impact on disease transmission, the virus-resistance gene(s) must spread within the vector population in the wild. For diseases such as dengue, where remarkably few competent vectors are required to sustain epidemic transmission [6], such a resistance gene would have to spread to a high allele frequency, so that practically all mosquitoes in the target population carried at least one copy. Unfortunately, insertion and expression of a transgene imposes a fitness penalty; this may be small, but will still tend to make the transgene decrease in frequency over time, even if a large number are initially introduced [7].

If infection were itself highly deleterious, resistance might be a positive fitness trait, perhaps enough to cause the resistance gene to spread to fixation. But the viruses carried seem to have remarkably little negative impact on the mosquito vector. An infected mosquito does not clear the virus and remains infectious for the rest of her life. So simply shortening the life expectancy of female mosquitoes is potentially an effective way to reduce transmission. A first step towards a genetic control strategy using this principle was recently achieved, using a pathogenic mutant version of the intracellular bacterium *Wolbachia pipiens*, which reduces the lifespan of mosquitoes that carry it [8].

If the resistance transgene will not spread through a population on its own, then further genetic tricks are needed to make it spread. Natural self-spreading genetic systems include obligate bacterial endosymbionts such as *Wolbachia* and selfish DNA elements such as active trans-posons. However, artificial versions of self-spreading systems have proved remarkably difficult to construct, although a demonstration of spreading in *Drosophila* of an artificial DNA element based on the *Tribolium castaneum* selfish DNA system *MEDEA* (maternal-effect dominant embryonic arrest) [9] is a very promising development.

Several questions remain regarding these self-spreading systems. 'Can we get them to spread?' is important, but so is 'Can we get them to stop?' Both *Wolbachia* and *Medea* are extremely difficult to remove from a target population after release - probably impossible in the case of *Wolbachia* - and also difficult or impossible to stop from spreading beyond the target population, perhaps even to all populations of
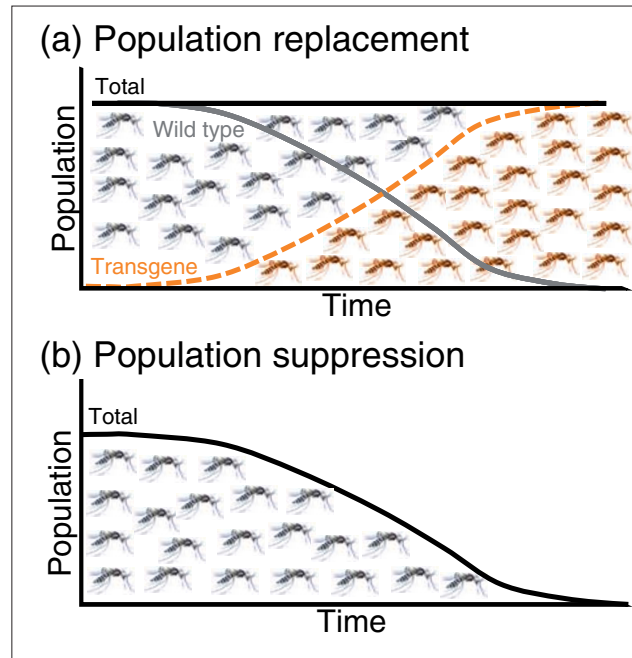
Figure 2
Population replacement and population suppression
(a) In population replacement strategies, the wild population is invaded by a heritable modification (e.g. transgene [5,9] or pathogenic *Wolbachia* [8]) that reduces the vector competence of the mosquitoes that carry it. The number of competent vectors therefore declines, but the total number of (female) mosquitoes remains relatively constant, though possibly with some transient change during the invasion. **(b)** In contrast, a population suppression strategy aims to reduce the total number of vector mosquitoes. The two panels illustrate the changes in female population number and type over time for the two strategies. In both cases the situation will eventually reverse due to various pressures such as resistance, mutation, immigration, etc, unless some maintenance activities are undertaken.

the species worldwide. This is new territory for genetic engineering and such use or outcomes may well be controversial. However, it is not an entirely new concept - analogies can be drawn with the introduction of exotic biocontrol agents, for which some of the same issues arise.

Population suppression using genetically engineered mosquitoes

The strategy outlined above is commonly known as 'population replacement': a wild vector population is converted to a modified one in which the mosquitoes have reduced vectorial capacity. The other main strategy for genetic control of mosquitoes is 'population suppression'. Here the objective is not to change the properties of the vector mosquitoes but to reduce their number, as in the case of the increased mortality induced by Cirimotich *et al.* [3].

This is a more familiar objective, in that it is also the aim of most source-reduction and chemical insecticide programs. The major current strategy in this area is based on the use of genetically sterile mosquitoes. In principle, large numbers of sterile male mosquitoes are released so that a wild female has a good chance of mating with a sterile male and so produces no or fewer progeny than usual. The population therefore tends to decline, and if enough sterile males can be released for long enough, the population collapses. This sterile insect technique (SIT) has been used for decades to control some major agricultural pests [10], sterilizing the insects by irradiating them before release. Applying conventional SIT to mosquitoes has proved problematic, but genetic modifications should be able to overcome many of the key difficulties and limitations. The leading genetically modified sterile release system, known as RIDL® (release of insects carrying a dominant lethal [11]), is ready to enter field trials for *Aedes aegypti*.

Genetics-based control systems share some attractive characteristics. They tend to be extremely species-specific, as the modified insects will mate only with their own species. The self-spreading systems are hard to develop but may be relatively cheap to deploy, as the genetic system does much of the work. Sterile-release methods such as RIDL® are relatively cheap to develop, but need regular releases of sterile insects to maintain sufficient sterile males in the field. This self-limiting nature - stop releasing and the transgene will rapidly disappear from the field population - may, however, be better accepted by the public and regulators, and these systems are likely to be the first ones used in the field.

None of these systems should be seen as a 'magic bullet'. Self-spreading systems will undoubtedly fail over time, due to mutation and pathogen evolution, and replacement versions will be required. Sterile-release methods will be much more effective in the context of an integrated vector management program than on their own. All of these methods will have to be tested in the context of different health systems, cultures and ecosystems; experience will determine where each is more or less valuable. Nonetheless, these powerful genetics-based vector-control tools, about to emerge from the laboratory into the field, provide rare new hope for the control, and perhaps one day elimination, of some of the world's major infectious diseases.

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