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# TRANSGENIC MOSQUITOES

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**Abstract** For transmission of dengue virus and malaria pathogens occur, the causing agents of these diseases have to complete their developmental cycle inside the mosquitoes *Aedes* and *Anopheles,* respectively. Mosquitoes are therefore potential weak links in dengue and malaria transmission. Genetically modified mosquitoes have been created aiming of either reducing their ability to transmit disease or suppressing target mosquito population by releasing transgenic males carrying a lethal dominant gene. There has been considerable progress over the last decade towards developing the tools for generating transgenic mosquitoes. These tools have provided researchers with the ability to engineer refractory mosquito vectors and transgenic mosquitoes carrying lethal genes, but there are fundamental gaps in our knowledge of how to transfer this technology safely and effectively into field.
**Key Words** Transgenic, suppression, replacement

## INTRODUCTION

Mosquitoes are responsible for the transmission of major human disease agents (Moreira et al., 2000). *Anopheles, Culex*, and *Aedes* genera include vectors for the three major groups of human pathogens: parasites of the *Plasmodium* genus, which cause malaria, filarids of the *Wuchereria* and *Brugia* genera and a variety of arboviruses, including the causing agents of dengue, yellow fever and West Nile (Atkinson and Michel, 2002; Wilke et al., 2009a).

The females of most mosquito genera need to take a blood meal in order to complete egg development. Repeated blood feedings over the life span of adult females make some of them transmitters of a wide variety of disease agents. Estimates from WHO (2008) indicate that there were 247 million malaria cases in 2008, leading to more than one million deaths, mainly among young children in sub-Saharan Africa (Atkinson and Michel, 2002; WHO, 2009). Worldwide morbidity and mortality from mosquito-borne diseases are substantial and on the rise. In addition, there are no effective vaccines or treatments for the most important vector transmitted pathogens (Atkinson et al., 2007).

Many developing countries, such as Brazil, have had large urbanization projects but without proper implementation of sanitation services. This recent expansion has increased the release of untreated effluents into the environment subsequently increasing the availability of breeding sites for a variety of mosquito species. The mosquito *Culex quinquefasciatus*, for instance, whose immature stages are able to survive in polluted water, has particularly benefitted and is rapidly increasing in numbers and distribution (Taipe-Lagos and Natal, 2003).

The extensive and frequent application of broad-spectrum pesticides to control insect vectors has resulted in the selection of resistant vector populations as well as raising environmental concerns stemming from their toxicity to non-target organisms (Muñoz et al., 2004). Additionally, some pathogens have evolved to become resistant to the prophylactics prescribed to treat the diseases they cause, as in the case of *Plasmodium* species. Increased global trade and travel, combined with increased tropical urbanization without the necessary improvements in urban hygiene, have also led to the resurgence of mosquito-borne diseases (Atkinson and Michel, 2002).

Given the failure of current methods to control the spread of these diseases, alternative methods of control are desperately needed, so considerable effort has gone into novel genetic mosquito control strategies (Atkinson et al., 2007). Substantial progress has been reached over the last decade towards developing the tools for generating transgenic mosquitoes, including germline transformation of the most important mosquito vectors of the *Anopheles*, *Aedes* and *Culex* genera, and the identification of promoters and effectors genes (Wilke et al., 2009b).

## GENETIC CONTROL

Genetic control of mosquitoes aims to achieve universal coverage by taking advantage of the male insect's efficiency in locating and mating with females of the same species. Control at the level of the insect vector can be divided into two categories.

The first one is called population replacement and it requires two components, a mechanism for pathogen resistance and a method to spread the gene into a population (Wilke et al., 2009b). This approach requires the use of a special genetic system capable of spreading the anti-pathogen gene of interest through a target vector population (Marrelli et al., 2006). Mechanisms of pathogen resistance have been developed in several mosquito species, for example RNAi to reduce transmission of dengue in *Aedes aegypti* (Franz et al., 2006), artificial peptides (SM1) to inhibit malaria development in *Anopheles stephensi* (Ito et al., 2002) and expression of cecropin to impair malaria development in *Anopheles gambiae* (Kim et al., 2004). Population replacement can also be classified as self-sustaining, since the transgenic releases need occur only once or a few times, and the construct will increase in frequency of its own accord and maintain itself at high frequency. Releases can often be of relatively fewer mosquitoes (inoculative releases) (WHO, 2009).

The second category is population suppression, which could be achieved by a technology known as 'release of insects carrying a dominant lethal gene' (RIDL) (Alphey, 2002) that is based on the sterile insect technique (SIT) (Knipling 1995). In RIDL, released transgenic males would mate with wild females and all progeny would inherit the lethal gene and consequently die, decreasing the target population (Alphey, 2002; Marrelli et. al., 2006; WHO, 2009). A wide range of effectors genes are available, all that is required is that they kill the cell when overexpressed (Alphey, 2002), or cause incapacitation of females in the field, being unable to attract and mate with males (Fu et al., 2010). Population suppression is a self-limiting strategy since repeated or recurrent releases are necessary to maintain the genetic construct in the target population. To have a significant epidemiological effect it will usually be necessary to release relatively large numbers of mosquitoes (inundative releases) (WHO, 2009).

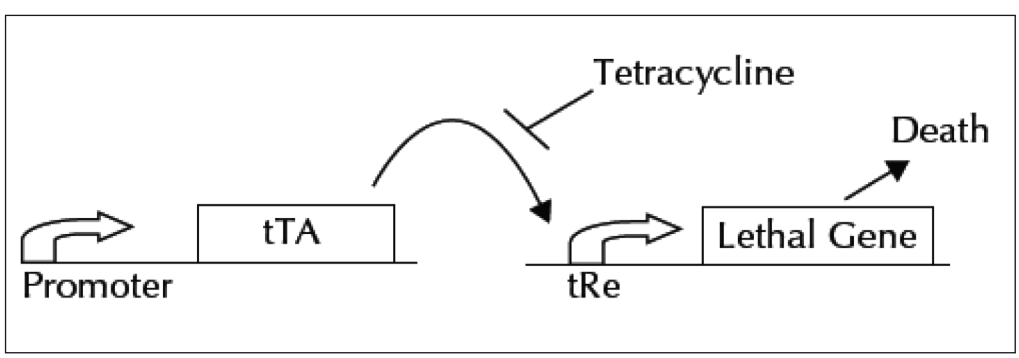


**Figure 1.** RIDL tetracycline-repressible system. The center of the system is the tetracycline-repressible transactivator protein (tTA) that is placed under the control of a promoter of choice. The selection of this promoter will determine the sex/stage-specificity of the system. When expressed, the tTA protein binds to a specific DNA sequence, tRe, driving expression from an adjacent minimal promoter. This promoter then activates the expression of a given sequence (the lethal effector gene), which is placed under its control. This gene will be expressed in the pattern of the promoter driving tTA. However, in the presence of low concentrations of tetracycline, the tTA protein does not bind DNA, and so expression of the lethal gene is prevented. Adapted from Alphey (2002).

## POPULATION REPLACEMENT - SELF-SUSTAINING

Population replacement requires two components, a mechanism for resistance and a method to spread the gene into a population (Wilke et al., 2009b). Transgenic mosquitoes carrying a gene that acts as an effector molecule interfering with a parasite or a virus development cycle are not expected to have a fitness advantage when compared to wild mosquitoes. Therefore Mendelian inheritance alone will not be able to spread the refractory gene into a population (Vernick et al., 2005). To overcome this issue, a gene drive mechanism has to be implemented (Sinkins and Gould, 2006).

Mosquito-borne diseases are propagated when the mosquito ingests an infected blood meal. The ingestion of blood leads to a dramatic activation of many genes encoding digestive enzymes (Moreira et al., 2000). Regulatory

regions of such genes can be used to express anti-pathogen effectors molecules in engineered vectors in a precise temporal and spatial manner, designed to maximally affect a pathogen (Kokoza et al., 2001). In insects, the fat body is a particularly important target for engineering anti-pathogen properties, because it is the most potent secretory tissue that releases its products to the hemolymph, which is a crossroad for the majority of pathogens (Kokoza et al., 2001). Thus the availability of gut-specific promoters to drive the expression of antimalarial proteins in the mosquito midgut would open a new frontier to hinder *Plasmodium* development in the mosquito (Moreira et al., 2000).

Population replacement is currently at the research stage of development and there are several major problems to overcome. There is currently no proven technique to drive a refractory gene into a population and there are many epidemiological and entomological risks that need to be assessed (Benedict and Robinson, 2003).

## POPULATION SUPPRESSION - SELF-LIMITING

For many insects the adult males are harmless, in this case, there is no imperative to kill all of the progeny of the released insects, and just killing all the female progeny is likely to be as effective, or possibly more effective (Alphey, 2002). Additionally, the use of a sex-specific promoter driving the expression of the repressible lethal gene can be used to halt the survival of one of the sexes (the one with the active sex-specific promoter) making automatic the separation of males and females and thus potentially reducing production costs (Muñoz et al., 2004).

Constructs should be engineered to reduce possible harm to the non-targeted subpopulation by choosing promoters with low leaky basal expression, and effectors proteins that act in a measured manner, rather than in a runaway, catalytic manner in which even small quantities of effector protein might be toxic (Marrelli et al., 2006).

For the purposes of mass rearing and release of the transgenic strain the dominant lethal must be repressible contingent on a permissive condition such as a food additive or an environmental variable absent in the wild but present in the rearing facility (Figure 1). Such a repressible RIDL system also serves to act as a failsafe for escapees since any accidental release of mosquitoes from a rearing facility are sterile without the repressor (Benedict and Robinson, 2003). One particular advantage of population reduction over population replacement is that the released individuals are sterile and do not spread any genes though a population. Inadvertent release of even millions of genetically sterile insects will only have a transient effect on the target population (Wilke et al., 2009b).

RIDL has the additional feature that the F1 males are themselves heterozygous for a dominant female-specific lethal and so half of their daughters die, and so on. This effect can be improved in several ways, most simply by making the released males homozygous for a dominant female specific lethal at more than one locus (Alphey, 2002). A population reduction method using a strain of *Aedes aegypti* homozygous for a dominant lethal genetic system is available for field use (Alphey and Andreasen, 2002; Alphey et al., 2002) and soon it will be a component of an integrated vector management strategy.

## PERSPECTIVES

Differently of other infectious diseases that are transmitted directly from person to person, human malaria and dengue are transmitted only via mosquitoes. In theory, any method of reducing or eradicating vector populations might have an impact in the disease transmission. The self-limiting (suppression) population method using strains of *Aedes aegypti* homozygous for a dominant lethal genetic system is now available for field use (Alphey and Andreasen, 2002; Alphey et al., 2002). These strains have been already tested in laboratory and contained "semi-field" conditions in Malaysia, Mexico and Cayman Islands (Wise de Valdez et al., 2011), and soon they could be components of an integrated vector management strategy. While population replacement strategies have shown several major problems to overcome, suppression strategies based on SIT are closer to reality. Therefore, the disease endemic countries need an international guidance to assess the risks and benefits of using genetically modified mosquitoes. Since they may reach different conclusions about using these mosquitoes the World Health Organization (WHO) and its partners are in process to provide best practice guidance to the endemic countries on these issues (Mosqguide) (Mumford et al., 2009). WHO has also held a special technical consultation on this research and in collaboration with the Foundation for the National has developed a series of planning meetings on Progress and prospects for the use of genetically modified mosquitoes to inhibit disease transmission (WHO, 2009).

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