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Control of vector populations using genetically modified mosquitoes

ABSTRACT

The ineffectiveness of current strategies for chemical control of mosquito vectors raises the need for developing novel approaches. Thus, we carried out a literature review of strategies for genetic control of mosquito populations based on the sterile insect technique. One of these strategies consists of releasing radiation-sterilized males into the population; another, of integrating a dominant lethal gene under the control of a specific promoter into immature females. Advantages of these approaches over other biological and chemical control strategies include: highly species-specific, environmentally safety, low production cost, and high efficacy. The use of this genetic modification technique will constitute an important tool for integrated vector management.

DESCRIPTORS: Mosquitoes, genetics. Animals, Genetically Modified, parasitology. Genetic Techniques, utilization. Mosquito Control. Review.

INTRODUCTION

We carried out a literature review in order to collect information on alternative forms of vector control, focusing on mosquitoes of the *Anopheles* and *Aedes* genera. A search in the PubMed basis was carried out using the following descriptors: Culicidae, (including *Anopheles*, *Aedes*); Vectors AND Control; Mosquitoes AND Transgenic; Mosquitoes AND SIT; Mosquitoes AND RIDL; Culicidae AND Control; Culicidae AND SIT; Culicidae AND RIDL. In addition, books, theses and government websites were consulted.

Mosquitoes (Diptera: Culicidae) have been the subject of intense study since the late 19th Century, when they were first linked to the transmission of pathogens to man and other vertebrates. The *Anopheles, Culex*, and *Aedes* genera include vectors for the three major groups of human pathogens: parasites of the *Plasmodium* genus, which cause malaria; filariae of the *Wuchereria* and *Brugia* genera; and a variety of arboviruses, including the causing agents of dengue and yellow fever.[24]

In the last century, during the 1950's and 60's, vector control programs in many countries were based on chemical strategies that made unrestricted use of insecticides such as DDT. These measures led to the successful eradication malaria from Southeastern Europe and Taiwan, and reduced the morbidity rate in India from roughly 75 million to about 100 thousand cases per year.[7] Prior to this effort, pyrethroid-based insecticides were intensely used in the 1930's to fight *Anopheles gambiae*, leading to the eradication of this species from Northeastern Brazil. Furthermore, the fight against *Aedes aegypti*, which in 1956 was considered as eradicated in Brazil, was based on an active search for potential breeding sites for this mosquito.[9]

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Beginning in the 1980's, successive dengue epidemics in Brazil revealed that reinfestation with *Aedes aegypti* had taken place throughout the country. Similar control measures are currently in place, but are less efficient now than they were in the 1950's. Amidst successes and failures, the use of chemical strategies for vector control has been the subject of intense criticism, aimed mostly at its effects in terms of environmental contamination, killing of non target organisms, and selection of insecticide-resistant mosquito populations.[9]

To achieve greater efficiency, and to preserve the environment from contamination, the bases for integrated pest management were established.[3] According to these guidelines, use of synthetic insecticides is limited to emergency situations, and biological control and environmental management are encouraged as priority strategies. Ecologic awareness among managers and scientists led to a search for alternatives that would not damage the environment. Anti-dengue campaigns in the late 20th Century already emphasized education and sanitation, and chemical control was restricted to epidemics. Entomopathogens of the *Bacillus* genus gained in importance in the biological control of vectors in several different programs.[a]

However, mosquito control measures have failed to achieve their goals, mostly because of the mosquito's great reproductive capacity and genomic flexibility.[23] These two characteristics are exemplified by two observations. The first is the rapid selection of lines resistant to chemical and biological insecticides used in vector control, as well as the emergence of resistance to different environmental conditions. The second is the existence of a variety of closely related species that form complexes of cryptic species, some of which seem to be undergoing speciation in the process of adapting to an environment modified by man.[4] Resistance to insecticides has led to serious public health problems, contributing to the resurgence of mosquito-borne parasitoses and arboviroses.

Recent attempts to use DDT in India were unsuccessful primarily because of the emergence of vector resistance. In Southern Africa, resistance to pyrethroids has led to a return to using DDT in households, which was followed by an increase in the number of cases of malaria.[10] In light of this type of problem, other strategies for mosquito control must be considered, including genetic control (use of sterile mosquitoes and related techniques). The sterile insect technique has been widely used, and has succeeded in controlling agricultural pests[6] and, in certain cases, mosquito vectors.

The aim of the present study was to revisit strategies of genetic control of mosquito vector populations.

THE STERILE INSECT TECHNIQUE

In 1955, Knipling[12] proposed the concept of introducing sterile insects into the population as a form of controlling pests with agricultural importance. According to Robinson (2002),[20] the sterile insect technique (SIT) is based on mass rearing, radiation-mediated sterilization, and release of a large number of male insects into a given target area. Released males will mate with wild females, thereby reducing the reproductive capacity of the wild population and reducing population size in subsequent generations. Releasing a sufficient number of sterile males for a sufficient amount of time can lead to the collapse of the target population and its consequent suppression in, or even total elimination from, the target area. This technique is thus species-specific, and does not damage the environment.

Genetic control aims to achieve universal coverage by taking advantage of the male insect's efficiency in locating and mating with females of the same species.[27] Resistance to insecticides among the target population is irrelevant to the success of this type of method. The possibility that wild females avoid mating with artificially raised or sterilized males is a point of concern. Nevertheless, there is a real possibility that the release of sterile mosquitoes may be able to eradicate isolated target populations, for as the density of the wild population decreases, the proportion of sterile mosquitoes in this population increases, favoring mating between sterile male and wild female.

The paradigm for this methodology was the successful elimination of *Cochliomyia hominivorax* (the causing agent of myiasis) from Southern United States, Mexico, and Central America. This area is currently protected from reinvasion from South-American flies by means of a barrier in Panama consisting of only a few sterile flies. Sterile flies were also used to eliminate the potentially devastating entry of this species into Northern Africa.[6]

There are other examples of eradication and control of agriculturally important pests and even of pathogen vectors using SIT, such as the eradication of the Tsetse fly, the vector of cattle trypanosomiasis (sleeping sickness) in Zanzibar.[26]

Sorting of males and the effects of radiation

One of the difficulties in implementing a program of sterile insect release is the need to release into the wild a pure male population. Manual sorting, in addition to being extremely labor-intensive, results in an unacceptably high level of female contamination among sterile males of certain species.[2,8] Systems capable of causing

[a] Cuba LM. Situação atual do controle biológico no manejo integrado de mosquitos (Diptera: Culicidae). [master's dissertation]. São Paulo: Faculdade de Saúde Pública da USP; 2005.

the death of females at any stage of their life cycles have thus been developed to automatically sort males from females, eliminating manual separation and reducing female contamination to very low levels.[22]

Sex determination in the fruit fly is heavily influenced by the sex chromosome system (XX/XY). A fly line genetically altered to allow for genetic sexing can be achieved by introducing a lethal autosomal recessive mutation in specific situations. For instance, in a mutant background sensitive to high temperatures, the resistant wild-type allele can be linked to the Y chromosome by translocation. The mutant line for genetic sexing will thus comprise females that are homozygous for temperature sensitivity and males that are phenotypically normal for this trait.[14] Systems that cause the death of females have the advantage of being applicable to the population as a whole, since individual manipulation of pupae may have a negative effect on the final quality of the batch of released males.[21]

The success of genetic sexing among flies is directly attributable to the fact that embryos can be easily subjected to high temperatures. A mutation sensitive to temperature and that is only expressed late in development will generate operational issues, given the unfeasibility of regulating the temperature of large volumes of food in large rooms.[15]

Adaptation of the sterile insect technique to mosquito vectors

The late 1960's and early 1970's saw a great deal of optimism with regard to the use of SIT as an alternative strategy for controlling mosquito vectors. However, SIT has been used only against a few species of mosquitoes to date, mostly because of certain fundamental problems inherent to the system.

Genetically modified males must be able to compete with their wild-type counterparts for mating opportunities. The process of sterilization, especially when based on irradiation, can lead to a drastic loss in mating capacity when compared to unmanipulated males. As well as being less competitive, irradiated males may also have a shorter life-span.[1] For example, a method used for sterilizing the fruit fly *Ceratitis capitata* leads to a four to ten-fold loss in competitiveness, which hamper any efforts to eradicate this species.[5,19]

In a study carried out in 1981 in California (USA), approximately 85 thousand irradiated *Culex tarsalis* males were labeled with a fluorescent compound and released into a semi-isolated canyon for monitoring abundance and sterility. Sterilized males dispersed satisfactorily, and accounted for 30% of all captured males. However, sterility levels reached only 11%, a level insufficient to suppress, or even cause any decrease, in the female population. The majority of irradiated males could not compete with wild males for mating with local wild females.[16]

Irradiation facilities are expensive and potentially dangerous.[1] There is also a risk of releasing individual insects that remained fertile in spite of irradiation, which is also potentially dangerous. Logistics are complicated by the need to release males at a predetermined stage of the life cycle as well as by the distance between irradiation facility and target area.

Another drawback of using SIT for mosquito vectors is that, from the ethical and safety perspectives, the release of sterile male mosquitoes is only acceptable if sterile male lots are not contaminated with females.[22] Male mosquitoes do not make blood meals, therefore being unable to transmit pathogens or cause distress. For *Culex* and *Aedes* mosquitoes, sexing can be done based on the size of the pupa.[2] This system has been used in India, and resulted in as few as 0.2% females being released among males. However, with production reaching hundreds of thousands of mosquitoes each day, large numbers of females were ultimately released into the environment, compromising the project's viability.[21]

Releasing females among the irradiated males leads to an increase in the number of females in the environment. In addition to causing distress and increasing disease transmission, this can also interfere with the mating of irradiated males to wild females due to competition.[14]

For *Anopheles* mosquitoes, mechanical sexing based on the pupa size is inefficient. In these cases, an alternative for producing males only is to induce sexual dimorphism using genetic strategies. Such methods are known as genetic sexing, and were described above. The most frequently used methods are based on radiation-induced Y-chromosome translocation of dominant selective markers complementing an X linked or autosomal trait, such as pupa color, temperature-sensitive lethality, or insecticide resistance.[19] However, as discussed above, the need for irradiation remains, resulting in loss of mating capacity among these insects when compared to wild males. Systems based on chromosomal aberrations tend to be unstable, and reversion may constitute a problem when releasing large numbers of insects. In addition to reducing insect fitness, this also makes mosquitoes less effective for SIT.[25]

TRANSGENIC INSECTS CARRYING DOMINANT LETHAL GENES

An offshoot of SIT, the release of insect carrying a dominant lethal gene (RIDL)[25] technique is a strategy that consists of suppressing reproduction of target insects by releasing into the environment transgenic insects that, when crossed to the former, will lead to a decline in the size of the wild population.[13]

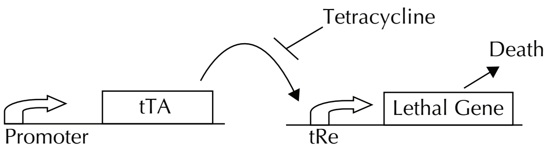

Figure. Tetracycline-repressible system. The tetracycline-repressible transactivator protein (tTA) is placed under the control of a promoter of choice. The selection of this promoter will determine the sex/developmental-specificity of the system. When expressed, the tTA protein binds to a specific DNA 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).1

The RIDL system proposed by Thomas et al[25] (2000) consists of introducing a lethal dominant gene under control of a female-specific promoter, such as that of vitellogenin. Expression of the lethal dominant gene can be inactivated by treatment with tetracycline, allowing a colony to be maintained. When male and female separation is required, tetracycline is removed from the system, causing the death of all females.

This system is centered on the expression of tTA, a fusion protein that combines sequence-specific tetracycline-repressible binding to tRe, a tetracycline-response element, to a eukaryotic transcriptional activator. In the absence of tetracycline, this protein will bind to the tRe sequence, activating transcription from a nearby minimal promoter.[1] A way of making this system female-specific is to place the tTA protein under control of a female-specific protein (Figure).

When preparing mosquitoes for release, the repressor is inactivated and the lethal gene is expressed, causing the death of all females. When mating with wild females, males homozygous for the lethal gene will produce heterozygous progenies, of which only males will survive.

Thomas et al[25] (2000) constructed a RIDL system in *Drosophila melanogaster* using transcriptional control elements to guide the expression of tTa. The protein was first expressed under control of the fat body activator *Yp3*, which promotes expression in female larvae and adults, but not in males.[25] Expression of the cytotoxic gene is thus expected to occur in the *Yp3* pattern, being lethal to females, and allowing only males to survive and to be released into the environment. This creates a genetic system for hereditary sexing.[25]

RIDL has certain advantages over SIT: insects produced are more competitive in the wild; there is no risk associated with radiation or with release of non-irradiated insects; it can be applied to insects that do not tolerate radiation; and it is not associated with high financial or environmental costs. The lethal gene is expressed according to the pattern of the promoter controlling tTA. In the presence of low concentrations of tetracycline, the tTA protein does not bind DNA, and expression of the lethal gene is prevented (Figure).

PERSPECTIVES

The RIDL system can function in other species of mosquitoes. It has been successfully applied to *Drosophila melanogaster*,[11,25] indicating that it can potentially be adapted to use in mosquitoes and other vectors of human pathogens. This system was recently adapted for use in *Aedes aegypti*, based on a non-female-specific construct (LA513)[18] that produces mosquitoes that die as larvae in the absence of tetracycline, but which can develop normally when raised in the presence of this drug. Furthermore, new genetic constructs have been proposed that rely on the use of a promoter specifically activated in immature *Aedes aegypti* females, known as *Act4*.[17] These systems have shown promising results in the laboratory, and field tests are likely to be carried out in Malaysia in the near future.

The RIDL system has countless advantages over other vector control systems, such as ease of colony maintenance and sexing, low production cost, and high efficiency. Though the actual potential of the RIDL system for mosquito control is unknown, the above-mentioned factors may make it an important tool for the integrated management of medically and agriculturally important pests in the near future.

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