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Towards mosquito sterile insect technique programmes: Exploring genetic, molecular, mechanical and behavioural methods of sex separation in mosquitoes☆,☆☆

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ABSTRACT

When considering a mosquito release programme, one of the first issues to be addressed is how to eliminate/separate the females. The greatest number of options might eventually be available for those who can use transgenic mosquitoes, but the inherent characteristics of the target species may also provide possibilities for interim measures until more efficient methods can be developed. Differences in intrinsic size, in behaviour and in development rate between females and males are often available and useful for sexing. Efficient species-specific systems for eliminating females at the embryo stage have been developed, but most have since been discarded due to lack of use. Ideal systems specifically kill female embryos using some treatment that can be manipulated during production. Such killing systems are far more efficient than using intrinsic sexual differences, but they systems require selectable genetic markers and sex-linkage created by rare random chromosomal rearrangements. While intrinsic sexual differences should not be considered as long-term candidates for the development of robust and efficient sexing approaches, in the absence of these, the accessibility and integration of less efficient systems can provide a stop-gap measure that allows rapid start up with a minimum of investment. The International Atomic Energy Agency is funding over a 5 year period (2013–2018) a new Coordinated Research Project on “Exploring Genetic, Molecular, Mechanical and Behavioural Methods of Sex Separation in Mosquitoes” to network researchers and to address the critical need of genetic sexing strains for the implementation of the sterile insect technique (using radiation-sterilised or transgenic male mosquitoes) and for insect incompatibility technique programmes against disease-transmitting mosquitoes.

1. Introduction

Among the major vectors of human diseases, mosquitoes are the most devastating ones (World Health Organisation, 2012). Urbanisation, globalisation and climate change have accelerated the spread and increased the number of outbreaks of new mosquito borne diseases, especially dengue (Kyle and Harris, 2008; Weaver and Reisen, 2010; Kilpatrick, 2011).

In view of the problems associated with conventional mosquito control, such as insect resistance and potential negative side-effects on human health related to insecticide use, great efforts are required to develop new or complementary control techniques for major mosquito species (McGraw and O'Neill, 2013), including the sterile insect technique (SIT). The SIT could become an increasingly

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important component of area-wide integrated vector management (AW-IVM) programmes for key insect vectors. With the increase in vector-borne diseases and their toll on human health and mortality, there have been recurring requests from International Atomic Energy Agency (IAEA) Member States to develop tools and techniques for the SIT, including the development of sexing strains to allow male-only releases which are essential for the effective application of the SIT to control mosquito populations. The SIT has the capability to suppress, or in special situations, to eradicate existing vector populations and to prevent population outbreaks and new establishments (Dyck et al., 2005).

Operational use of the SIT for other insect pests continues to reveal areas where new technologies could further improve efficiency and thus lead to more efficacious programmes. There are many aspects of a mosquito SIT package that require improvements to move to the operational level, e.g. improved mass rearing and release technologies, quality control, field monitoring, etc. However, one critical area, where important advances need to be made before any SIT application is possible, concerns the development of genetic sexing strains (GSS) for the elimination of females from the mosquitoes that are released. Unlike agricultural pests where the release of both sexes is primarily of concern for economic reasons, in mosquitoes it is an essential prerequisite to release only males since females are blood feeders and transmit disease. Mosquito SIT thus depends on reliable elimination of females.

Currently, area-wide integrated pest management (AW-IPM) programmes with an SIT component have been successfully implemented for several very important fruit fly and lepidopteran species where the development of improved strains, especially GSS (in the case of fruit flies), led to major enhancements in SIT applicability and efficiency (for a list of the species currently targeted for SIT programmes see: http://nucleus.iaea.org/dirsit/DIRSITx.aspx). The experience gained from these programmes primes the new Coordinated Research Project (CRP) for the development of sexing strains in mosquitoes. There are several mosquito species that are vectors of different pathogens, each with a particular distribution. Based on the severity of the disease, the requests from IAEA Member States and the availability of scientific tools and information, initial efforts to develop mosquito sexing strains should focus on: *Anopheles arabiensis* (vector of malaria), *Aedes albopictus* and *Ae. aegypti* (both vectors of arboviruses including dengue and chikungunya).

The development of mosquito sexing strains can be achieved using different approaches, but they all rely on a stable genetic change introduced and maintained in the strain developed for release. Genetic changes can be introduced either through chemical mutagens, irradiation and classical genetics or modern biotechnology, specifically genetic transformation. Each approach has advantages and disadvantages, especially in relation to transferability of systems between species, stability in mass-rearing conditions and regulatory approval. A CRP has been initiated by the FAO/IAEA with the title “Exploring Genetic, Molecular, Mechanical and Behavioural Methods of Sex Separation in Mosquitoes”, which over five years will bring together collaborators to work towards the development of effective sexing mechanisms. Through annual meetings, participants will share results and experiences to optimise the chances of new technologies, equipment or techniques being developed. As the title suggests, sex separation methods based on physical, mechanical and behavioural approaches will also be considered during the course of this CRP. However, it should be noted that these alternative methods are currently appropriate only for small scale operations, and there is a need to develop state-of-the-art sexing strategies based on genetic and molecular approaches for large scale SIT and related mosquito release programmes which will be crucial for long term applications.

Given the necessity of sex separation – more specifically female elimination – one would expect that the variety of genes with tissue, stage and sex-specific expression would have resulted in female-elimination being one of the first fruits of mosquito transgenesis. Various approaches for accomplishing this have been described previously (Papathanos et al., 2009; Nolan et al., 2010), but they have not been easily realised in spite of early demonstrations of feasibility in *Drosophila melanogaster* (Heinrich and Scott, 2000; Thomas et al., 2000). Sex-specific fluorescent transgenic markers for the screening of males using automated sorters have been developed (Catteruccia et al., 2005), and improvements in this field show their high specificity (Marois et al., 2012). The robustness of such approaches in routine production settings or high volumes has not been determined yet and awaits testing (Knols et al., 2006).

Because of yet unrealised transgenic embryonic female elimination technology for mosquitoes and the fact that some programmes require, or prefer, non-transgenic mosquitoes, recent SIT programmes have been forced to rely on long-established physical size separation methods for selecting the smaller male *Aedes* spp. pupae (Bellini et al., 2007; Harris et al., 2012; O'Connor et al., 2012). Without this relatively simple method, none of these programmes would be possible. These examples attest to the value of continued use and development of methods that exploit the intrinsic differences between females and males. These, and similar methods, have been described in detail previously (Papathanos et al., 2009), thus in the following section, we discuss briefly what has been accomplished using these strategies and suggest how they might be improved, particularly using novel unexplored approaches.

2. Genetic sexing strains (GSS)

2.1. *Development of genetic sexing strains based on irradiation and classical genetics*

The development of sexing strains has been a catalytic factor for both the technical and the economic feasibility of the SIT as an integral component of AW-IPM programmes. One example of how insect strain improvement can significantly enhance SIT applicability and efficiency has been the use of a GSS in the Mediterranean fruit fly (medfly), *Ceratitis capitata*, AW-IPM programmes, a technology developed through the Agency's CRP programmes with support from the FAO/IAEA Agriculture and Biotechnology Laboratories in Seibersdorf, Austria. A series of strains have been developed that progressively increased the stability and specificity of these strains which are used worldwide.

2.1.1. *The medfly GSS as a case-study*

Typically, a GSS consists of at least two principal components: (a) a selectable marker which is necessary for sex separation or female killing and (b) a Y-autosome translocation, T(Y;A) which is required to link the inheritance of this marker to sex (Franz, 2005). In insects with homomorphic sex chromosomes such as *Ae. aegypti*, the translocation must be to the male-determining factor. According to Franz (2005), the selectable marker has to fulfil several criteria that determine the overall efficacy of the sexing strategy. Preferentially, the selectable marker should kill females at an early developmental stage, preferably at the embryonic stage, by physical or chemical means. It has been shown in medfly that killing females at the embryonic stage is a very robust and efficient method and at the same time significantly minimises the costs required for the rearing and elimination of the females. In addition, the use of chemicals for selection raises concerns for some regarding its effect on the insect itself (e.g. chemicals in the diet may affect the symbiotic communities) as well as on the waste disposal and environmental protection. These concerns must be addressed and any safety risks managed. In any case, the viability and the productivity of the