Entomologia Experimentalis et Applicata

DOI: 10.1111/eea.13085

MINI REVIEW

Sex separation of *Aedes* spp. mosquitoes for sterile insect technique application: a review

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Accepted: 11 May 2021

Key words: area-wide integrated vector management, AW-IVM, classical genetics, control methods, Culicidae, Diptera, genetic sexing strains, GSS, separation of sexes, sterile insect technique, mosquitoes

Abstract Separation of the sexes is necessary for the application of the sterile insect technique (SIT) in mosquitoes due to the hematophagous habits and disease vector activity of the females. In this review we analyze the history, current status, and future perspectives for the development of genetic sexing strains (GSS) of *Aedes* mosquitoes (Diptera: Culicidae). Various genetic control methods for mosquitoes are reviewed, as are their need for sex-separation methods. We focus on areas of opportunity where GSS developed with classical genetic methods can be used. Regulatory restrictions and social acceptance of various control methods are analyzed. We conclude that the development of GSS by classical methods represents the most viable option for separation of the sexes and the application of large-scale SIT programs within an area-wide integrated vector management (AW-IVM) approach.

Introduction

Mosquitoes of the genera *Culex*, *Anopheles*, and *Aedes* (Diptera: Culicidae) are well known as vectors of diseases affecting humans and other vertebrates. Certain mosquitoes transmit pathogens and arboviruses causing diseases in humans that are considered global public health problems (Onchuru et al., 2016). These vectors are established in urban, suburban, and rural environments. Their broad geographic distribution and the ecological niches they occupy make them a serious threat to human health (Tandina et al., 2018).

Aedes aegypti (L.) and *Aedes albopictus* (Skuse) are the most prominent species within the *Aedes* genus in terms of public health threats. *Aedes aegypti* is the main vector of the viruses that cause dengue and yellow fever (Failloux et al., 2002), and recently chikungunya and Zika (Fernández-Salas et al., 2015; Shragai et al., 2017). *Aedes albopictus* is considered another important vector species of these viruses, especially in peri-urban and rural areas (Reiter et al., 2006; Delatte et al., 2008; Paupy et al., 2010). The vector role of both species is reflected in the recent outbreaks of Zika in 2015 and 2016, during which regions of Latin America and the Caribbean were severely affected by the presence and coexistence of these vectors in peri-urban spaces (Capurro, 2018).

Despite advances in the development of vaccines against certain arboviruses – such as dengue, Japanese encephalitis, tick-borne encephalitis, and chikungunya (Monath, 2013) – there are still no effective vaccines against these diseases. The cornerstone of public health efforts remains the efficient control of vector insects in order to avoid the emergence and reduce the spread of arboviruses (Arredondo-García et al., 2016).

To avoid the emergence and reemergence of vector-borne diseases, interest has focused on the search for alternative strategies to the current conventional control and suppression methods. Additional methods such as the sterile insect technique (SIT), the incompatible insect technique (IIT), and transgenic approaches have become

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consolidated and are increasingly effective (Alphey et al., 2010; Yamada et al., 2012; Zacarés et al., 2018). Based on successes achieved with various pest species, the SIT, also known as autocidal control, is a widely accepted strategy as part of an area-wide integrated pest management (AW-IPM) approach, as it has no adverse effects on the environment and is specific to the target pest (Dyck et al., 2021). The technique is based on the principle of reduction of reproductive potential and involves the production and systematic release of millions of sterile male insects that compete with wild males to mate with wild females, causing a population decline over time (Knipling et al., 1968). Application of the SIT to suppress mosquito vectors requires an effective method for separation of the sexes in order to eliminate the females and release only males. Given that females are blood-sucking and can therefore transmit diseases, it is necessary to have a method that ensures a high level of recovery of males with little or no contamination by females (Mains et al., 2016). In small-scale rearing processes, this requirement has been moderately achieved by mechanical separation methods through systems of glass plates or sieves designed to separate males and females according to sexual size dimorphism in the pupal stage (Fay & Morlan, 1959). However, it is desirable to develop methods of separation of the sexes that are applicable under large-scale massive rearing conditions, which would facilitate the separation process and avoid the release of females. The acceptable level of female contamination will be almost null when dealing with disease vectors, whereas when dealing with mosquitoes as a nuisance because of their biting, a higher female contamination could be acceptable.

In this context, our aim here is to review the state of the art in the development of sex-separation methods for SIT application in *Aedes* mosquitoes and analyze future perspectives. First, we briefly describe current genetic control methods for mosquitoes, with an emphasis on the SIT. Then we review the history, principles, and practicability for implementation of available sex-sorting systems. We pay special attention to the advances made in the development of genetic sexing strains (GSSs), considering the approaches of classical and molecular genetics. Finally, we discuss the regulatory restrictions and social acceptance of these technologies, their strengths and limitations, and future perspectives.

Control methods

Methods used for mosquito control can be divided into four categories: (1) environmental management or sanitation (CDC, 2010), (2) chemical control (WHO, 2003; Baldacchino et al., 2015), (3) biological control (WHO/EMRO, 2003), and (4) genetic control (Wilke et al., 2009). Genetic control has been defined as "the use of any condition or treatment that can reduce the reproductive potential of noxious forms by altering or replacing the hereditary material" (WHO, 1964). These genetic control methods follow two approaches: population suppression – reducing the total population (infected / uninfected) and thus, the probability of human-vector contact – and population replacement – substituting the population that can transmit a pathogen (susceptible strain) with individuals that cannot transfer the pathogen (refractory strain) (Curtis & Graves, 1988). For these approaches to be successful, it is necessary that the population of infected vectors be below a given threshold, so that the probability of transmission decreases (Terenius et al., 2008).

The sterile insect technique (SIT), the incompatible insect technique (IIT), and some transgenic approaches achieve suppression of vector insect populations. These control methods require the release of only males, with the aim of reducing population growth and avoiding the release of hematophagous females (Bourtzis & Tu, 2018).

SIT: recent advances for genetic control of *Aedes* mosquitoes

After the first successful case of the SIT used against the screw worm *Cochiomyia homnivorax* (Coquerel) in 1954, application of the SIT has been extended to other pests of agricultural or public health interest (Dyck et al., 2021), such as mosquito vectors of diseases. Early attempts to establish programs of release of sterile mosquitos have taken place since the 1950s with the genera *Culex* (Laven, 1967, 1971; Laven & Aslamkhan, 1970; Patterson et al., 1970; Grover et al., 1976; Curtis et al., 1982), *Anopheles* (Weidhaas et al., 1962, 1974; Davidson et al., 1970; Lofgren et al., 1974; Bailey et al., 1980), and *Aedes* (McDonald et al., 1977; Petersen et al., 1977).

Given a lack of knowledge regarding the biology and ecology of the vectors and the absence of a sexing system, most of these experiments presented technical faults that impeded the success of the release programs (Benedict & Robinson, 2003). However, other early attempts did demonstrate efficacy in terms of suppressing vector populations (Laven, 1967; Breeland et al., 1974; Breeland, 1974; Lofgren et al., 1974; Weidhaas et al., 1974).

Since the beginning of the first SIT attempts, innumerable studies have focused on the fulfilment of the requirements and the improvement of the methods and techniques for its successful application (Klassen et al., 2021). Past experiences marked the standard for the planning and design of future programs targeted at mosquito vectors. A technological package was also developed for the processes of mass rearing, sterilization, release, and quality control of sterile *Ae. aegypti* and *Ae. albopictus* (WHO/IAEA, 2020). This technological package describes the procedures applied in the Insect Pest Control Laboratory (IPCL, Seibersdorf, Austria) of the FAO/IAEA. The package includes guidelines for colonization and colony management, mass rearing, sterilization, and mark-release-recapture for estimation of populations. Complementary to this, new guidelines addressing transport and quality control, are being prepared. These guidelines are available online at: http://www-naweb.iaea.org/nafa/ipc/public/manuals-ipc.html.

To address the interest of UN member states, the WHO/TDR and the FAO/IAEA jointly published the 'Guidance Framework for Testing the Sterile Insect Technique as a Vector Control Tool against *Aedes*-Borne Diseases' (WHO/IAEA, 2020). Currently, pilot tests are being developed addressing the use of the SIT in Brazil, Cuba, Malaysia, Mexico, and USA for the management or suppression of *Ae. aegypti*, and in Thailand, Singapore, France, Germany, Greece, Italy, Mauritius, and Spain against *Ae. albopictus* (WHO/IAEA, 2020).

Incompatible insect technique (IIT)

The IIT is based on SIT but focuses on the suppression of populations through genetic mechanisms that induce sterility, such as cytoplasmic incompatibility (CI) (Knipling et al., 1968; Lees et al., 2015). Cytoplasmic incompatibility is caused by *Wolbachia*, a Gram-negative bacterium of the class α-Proteobacteria of the order Rickettsiales that mainly inhabits the somatic and reproductive tissues of its host (Werren et al., 1995; Dobson et al., 1999). Transmission is maternal and its presence causes different reproductive alterations, such as parthenogenesis in species of the order Hymenoptera (Stouthamer et al., 1993) and feminization in isopods (Crustacea) (Rigaud et al., 1991; Rousset et al., 1992; Juchault et al., 1994). It is an androcide (male-killing) in species of Coleoptera (Hurst et al., 1999; Fialho & Stevens, 2000), Lepidoptera (Jiggins et al., 2000), Diptera (Hurst et al., 2000), and Dromopoda (pseudoscorpions) (Zeh et al., 2005). *Wolbachia* infection results in CI (Hoffmann & Turelli, 1997) in arthropods (Wade & Steven, 1985; Breeuwer et al., 1992; O'Neill et al., 1992; Bourtzis & O'Neill, 1998). Cytoplasmic incompatibility is manifested by embryo mortality, which occurs when *Wolbachia*-infected males mate with non-infected females (unidirectional incompatibility) or with females that carry a strain of *Wolbachia* that is incompatible with that of the males (bidirectional incompatibility) (Araújo et al., 2015; Mateos et al., 2020).

Three approaches have been developed with regard to the introduction of *Wolbachia* in populations of vector mosquitoes. One approach has been the manipulation of survival of the vector. Brownstein et al. (2003) suggested that introduction of the *Wolbachia w*MelPop strain, originating in *Drosophila*, to *Ae. aegypti* reduced the longevity and fecundity of this mosquito. McMeniman et al. (2009) reported that the life expectancy of females infected by *Wolbachia w*MelPop-CLA *strain was reduced by up to 50%, favoring the interruption of dengue virus transmission. A second approach has been the protection against pathogens based on antiviral effects. Moreira et al. (2009) reported that the presence of the Wolbachia w*MelPop-CLA *strain, introduced to Ae. aegypti*, reduced the capacity for infection with dengue, chikungunya, and *Plasmodium* sp. A third approach has been the combination of SIT and IIT to reduce the risk of virus transmission by females that can be accidentally released and reduce the risk of population replacement (Lees et al., 2015). The viability of the combination of using irradiation (40 Gy dose) and CI was evaluated in pupae of *Ae. polynesiensis* (Marks) infected with *Wolbachia* (Brelsfoard et al., 2009). The results supported this approach as a preventative measure against the accidental replacement of the target population, as neither the biological attributes of the male insects or the CI caused by the presence of the bacteria were adversely affected.

Recently, Zheng et al. (2019) evaluated three lines of *Ae. albopictus* from Guangzhou, China. The first line carried a triple infection caused by artificial transfection of the native strain of *Wolbachia* (*w*Pip) of *Culex pipiens* L. to *Ae. albopictus*. After the artificial infection, crossing was conducted in which the females mated with wild males that presented natural superinfection by native strains of *Wolbachia* (*w*AlbA, *w*AlbB) to generate the line HC (*w*AlbA, *w*AlbB, and *w*Pip) that expressed high CI. The second line presented a natural superinfection by native strains of *Wolbachia* (*w*AlbA, *w*AlbB). The third was not infected. It appeared that infection by *Wolbachia* did not significantly affect the fitness of any of the mosquito lines and the HC strain of mosquitoes had potential for mass rearing and subsequent application in a combined IIT-SIT strategy. Following the generation and characterization of the HC line, Zheng et al. (2019) tested the IIT strategy. They released millions of incompatible HC sterile mosquitos in the field over a 2-year period. At the end of the study, almost 100% suppression of two wild populations of *Ae. albopictus* was achieved, and the mosquito biting rates were reduced by 88.7–96.9% in two study areas, demonstrating that the application of the combined SIT-IIT strategy was successful for the control of this vector.

Transgenic approaches

Genetic engineering has been used to produce genetically modified or transgenic insects that can be used to suppress populations. This approach can be classified into three types: (1) release of insects carrying dominant lethal genes (RIDL), (2) RNA management, and (3) use of homing endonuclease genes (HEG).

The mode of action in the RIDL approach is similar to SIT. In this case, males carry transgenes in their genome, which, after release, are transmitted to the wild females through copulation. The female offspring will be affected in terms of their biological attributes, such as their flight ability, thereby limiting their ability to search for food or mates (McGraw & O'Neill, 2013). Another approach utilizes transgenes that induce mortality. These are of late action and are expressed in the larval stage or in the early phase of the pupae (Phuc et al., 2007).

In RIDL, the genetically modified mosquito remains in the environment for a very short period. Currently, RIDL is the only available strategy for genetically modified mosquitoes. This approach has been strengthened through the development of guidelines for the direction of field trials with genetically modified mosquitoes (Benedict et al., 2008). Initially, RIDL was tested in several experiments in field cages with mixed results and it was found that field trials provided valuable information on the behavior of genetically modified mosquitoes (Fachinelli et al., 2013; Lee et al., 2013; Madakacherry et al., 2014). In the same way, genetically modified sterile male *Ae. aegypti* mosquitoes have been field tested in regions of Malaysia (Lacroix et al., 2012), the Cayman Islands (Harris et al., 2011, 2012), and Brazil (Malavasi, 2014).

The second strategy centers on the first stages of development of the mosquito. Through RNA management, the RNAi technique confers immunity to the individual through the construction of an inverted repeat of a genomic RNA of the invading virus, through a protection response that unlocks the double-stranded RNAi to avoid development of the virus (McGraw & O'Neill, 2013). One of the advantages of this focus is that these genetic constructions can be developed for other viruses of global importance, such as the West Nile virus (Arjona et al., 2011). Nevertheless, this approach is currently one of the least developed.

Finally, the use of homing endonuclease genes (HEG) is the third approach, based on cutting specific DNA sequences of ca. 30 bp. In an organism, that is heterozygous for the EG, the endonuclease cuts the intact copy of the recognized sequence in the chromosome that does not contain the HEG. Another function of the HEG is suppression of the population directed at the genes that alter biological processes such as fecundity, survival, and sexual proportion (Deredec et al., 2011). To date, HEG have been successfully introduced into species such as *Ae. aegypti* (Traver et al., 2009) and *Anopheles gambiae* Giles (Windbichler et al., 2011).

The need for a method of sexual separation: genetic sexing strains

In SIT programs, some IIT applications and some transgenic applications require the separation of the sexes, to avoid the release of females. Efforts to develop sex separation methods through molecular and genetic mechanisms have intensified. The challenges presented by SIT-based programs lie in maintaining the males in optimum conditions in order to secure adequate sexual performance in the field, despite various handling processes including the separation of the sexes (Crawford et al., 2020). As a result of these efforts, sex separation has benefited from the successful development of genetic sexing strains (GSS) for 19 pest species, but only seven of these can be mass-reared for SIT application. Of these seven GSS, two are mosquito species (*Anopheles albimanus* Wiedemann and *Anopheles arabiensis* Patton), and five are tephritid fruit fly species: *Anastrepha ludens* (Loew), *Bactrocera curcubitae* (Coquillet), *Bactrocera dorsalis* (Hendel), *Bactrocera tryoni* (Froggatt), and *Ceratitis capitata* (Wiedemann) (DIR-SIT, 2020). *Ceratitis capitata* is considered a model for the other species, given its stability for use in operational programs over prolonged periods of time (Robinson, 2002; Franz, 2005).

The FAO/IAEA joint division has implemented coordinated research projects on the subject (Lees et al., 2014). In the case of mosquito vectors, efforts to develop GSS have been redoubled for their subsequent implementation in programs that apply the SIT. Munhenga et al. (2016) evaluated the competitiveness of the GAMA strain of *An. arabiensis* under laboratory and field conditions, considering different proportions of sterile males (GAMA strain), fertile males (AMAL strain), and wild females (AMAL strain). They observed that the GAMA strain of *An. arabiensis*, at a proportion of 3:1 (3 GAMA males: 1 AMAL male), could compete successfully in terms of mating with wild females.

For the genus *Aedes*, using a combined SIT/IIT approach in *Ae. aegypti* in Thailand, Kittayapong et al. (2018) demonstrated that with the adjustable glass plates, 99% of the males were obtained for sterilization and release in the field, with only $0.06 \pm 0.10\%$ of contamination by females. Lebon et al. (2018) developed the first GSS for *Ae. albopictus*. This strain, known as Tikok, was created through a translocation that conferred resistance to dieldrin (rdl^R). These authors observed that the males have parameters that are acceptable for implementation of the SIT, including survival of the larvae and separation of the males with 98% efficacy. However, it will be necessary to conduct further studies in order to improve the percentage of larval eclosion.

Gunathilaka et al. (2019) used one behavioral method (double feeding with insecticide) and two mechanical methods (standard sieving and the Fay-Morlan glass plates method) for sex separation in each developmental stage of *Ae. aegypti* and *Ae. albopictus*. The standard sieving was 73 and 69% effective for *Ae. aegypti* and *Ae. albopictus*, respectively. With the mechanical glass separators, the efficiency values were 99% for both *Ae. aegypti* and *Ae. albopictus*, with 16 and 12% female contamination, respectively. The double feeding method with ivermectin and spinosad resulted in 100% females eliminated, showing the greater efficacy of this method compared to the mechanical methods.

Recently, Crawford et al. (2020) during the program Debug, developed technology focused on the mechanization of the sexual separation process as one of the main objectives of the project of suppression of *Ae. aegypti* in California, USA. This process was based on a three-step system that allowed a reduction in contamination by females during mass-rearing of *Ae. aegypti* transfected with *Wolbachia*. In the first step, the mosquitos are separated using sexual dimorphism by passing through an automated sieve, which achieved elimination of 94.9% of the females. In the second step, during the emergence of adults from the sieved male pupae in step one, individuals are inspected and labeled using images with industrial image analysis software, with around 95.6% of the males passing to the next phase. Finally, the images obtained during the inspection are transferred for correct identification under the judgement of five reviewers. If any female individual is detected, the release tube is purged. Final contamination by females is estimated at one female individual for every 900 million males with a cytoplasmic incompatibility (CI) of 95%. The project caused a reduction of 99% in the *Ae. aegypti* population over an area of almost 300 ha (Crawford et al., 2020).

These results indicate the effectiveness of the SIT and the feasibility of the mechanized method of sex separation. However, implementation of this technology requires an analysis of its costs and practicality on a larger scale. The use of mechanical and behavioral methods, or their combination during different developmental stages of mosquitoes under the SIT/IIT approach represents an efficient alternative for female elimination, with an acceptable yield of males and low contamination by females. This contamination by females can be as high as >10% or as low as <0.1%, depending on factors such as the ability and skills of the technicians and the rearing conditions (Zacarés et al., 2018). The current levels of contamination by females are unacceptable (Kittayapong et al., 2018), and these combined methods represent a viable option for small-scale projects until other more effective methods are developed.

Regulatory restrictions and social acceptance

New technologies for the control of mosquitoes represent a great challenge for researchers, due to the rift that exists in terms of establishing a balance between the scientific and social spheres. In general, the rejection of transgenic organisms by society is due to their possible negative effects on human health and the environment (Skerritt, 2000). However, risk assessment is necessary in order to determine their safety and efficacy, providing evidence and defining the technical requirements of the regulatory processes necessary to conduct such assessment under field conditions.

In this context, there is a series of regulations and limitations clarifying what this approach must address from different perspectives, evaluating the negative aspects, expected benefits, and autonomy of the project (Macer, 2005). Decision makers, organizations of joint projects, and collaborators all face the challenge of preliminary requirements to evaluate and anticipate ethical, social, and cultural aspects, for which reason the project must be structured through rigorous planning, including: (a) justification of the choice of area in which to develop the study, which involves an initial approach in order to establish a connection between the decision makers and collaborators of the participating communities; (b) evaluation of potential risks based on the regulations; and (c) the development of strategies of regulatory supervision for effective monitoring during field release tests (Lavery et al., 2008). Considering this aspect, it is possible to determine whether the program complies with the ethical aspects prior to conducting a release; i.e., that the program: (a) presents no ecological risks, (b) has the informed consent of the community inhabitants, and (c) does not compromise human health through the use and release of GM insects. Before any release of GM vectors into the field, it is important to determine the characteristics of the region, as the approach with the inhabitants represents a complicated task to tackle due to certain cultural and educational limitations. Therefore, the active participation of researchers together with the region's leaders represents an aid and link in the epidemiological work of GM mosquito management (Favia, 2015). However, it is vital to establish minimum standards of risk evaluation, as well as ethical principles, in order to determine the extent to which a program exceeds the permitted limit (Reeves et al., 2012). The use of transgenic mosquitoes can face strong opposition from public opinion and from opponents who severely criticize the release of genetically modified (GM) insect vectors in the environment, which brings the bioethics of these projects into question. In 2009, the small-scale release of transgenic mosquitoes was announced on Grand Cayman Island. This provoked controversy and divided opinions within the group of scientists dedicated to testing GM vectors (Enserink, 2010; Subbaraman, 2011).

In Mexico, Ramsey et al. (2014) developed a regulatory structure for working with genetically modified *Ae. aegypti* under field conditions, according to the guidelines of: (1) the National Intersecretarial Commission for Biosafety of Genetically Modified Organisms (CIBIOGEM), which is the federal agency that regulates the release of genetically modified organisms (GMO), and (2) the National Institute of Public Health / Regional Center for Public Health Research (INSP/CRISP), as well as (3) the Rio Florido community council, Chiapas, Mexico, as the regulatory organ for making community decisions. Following compliance with the regulatory, social, and infrastructural requirements, testing was initiated under field cage conditions (Facchinelli et al., 2013).

Despite advances in the regulatory framework for this specific project, the technique has not been upscaled for wide application. A limiting factor is the lack of a general clear regulatory framework regarding the release of GMO in Mexico and many developing countries (Quemada, 2016). Another limitation has been social and political opposition due to established prejudices and concerns on the ecological implications, considering that mosquito dispersal cannot be controlled (Handler, 2002).

Future perspectives

Efficient methods for sex separation still represent a major challenge for large-scale application of the SIT for *Aedes* spp. suppression. It is therefore vital to develop and characterize efficient mechanisms for sex separation – the two approaches currently available (classical genetics and transgenics), both present strengths and weaknesses.

The GSS currently established in SIT operational programs for other insect pests have been developed using classical genetics approaches, based on conditional or visible mutations that serve as selectable genetic markers, as well as chromosomal reordering (translocations linked to the male). In this way, a pseudo-sexual dimorphism is generated, in which the males are heterozygous and the females homozygous for the mutation, enabling separation of the males. Mutations such as genes with potential use in GSS have been evaluated; these express a visible phenotype, such as: pupal color (Rössler, 1979), wing morphology (McCombs & Saul, 1992), egg color (Tazima et al., 1951), and eye color (Rössler & Rosenthal, 1988). In addition, there are those that confer a conditional lethal effect: resistance to insecticides (Seawright et al., 1978), sensitivity to temperature (Franz et al., 1996), and lethal recessives (applied to ZW sex chromosome systems) (Marec, 1991). The Mediterranean fruit fly, *C. capitata*, is a model organism that presents a genetic sexing system comprising two genetic markers (sensitivity to temperature and pupal color) that can be applied to the separation of females during the immature stages. It is also one of the strains in which constant innovation is possible in the genetic construction of its sexing strains, in order to optimize the mass rearing and release processes (Franz, 2005; Meza, 2020).

Development of GSS for mosquito SIT using natural mutants and classical genetic approaches need to overcome the following disadvantages: (1) delay in the analysis of mutations and strains for isolating a suitable genetic marker, (2) effort required to translocate the selectable marker to the Y chromosome, (3) lack of guaranteed success due to unpredictability, and (4) reduced sexual performance of the mass-reared males (Papathanos et al., 2018). In contrast, the transgenic approach has the following advantages (a) existence of broad knowledge in relation to the formal genetics of *Ae. aegypti* and *Ae. albopictus* (Craig & Hickey, 1967a,b), (b) elimination of females in the early stages of development, which generates significant savings in terms of production costs over the course of an action program, and (c) the possibility of releasing males in the pupal stage for subsequent emergence of adults (Papathanos et al., 2009). However, after exploring the use of molecular, mechanical, and behavioural approaches, as well as those of classical genetics to achieve sexual separation in mosquitoes, it was concluded that classical genetic methods were the best option, as these can be used without regulatory restrictions. In contrast, the release of transgenic strains in SIT programs, despite notable advances, remains strictly regulated (Gilles et al., 2014).

Conclusions

The current SIT technological package developed for the suppression of *Aedes* mosquitoes provides a solid framework for the application of SIT within an area-wide integrated vector management approach. The only limiting factor for operational use on a large scale is the practical challenge of removing females following mass-rearing. Progress has been made in the development and characterization of GSS through classical genetic methods and it seems possible to produce strains in which females can be separated in large numbers and with a high level of accuracy (>99%). Considering the social, ethical, and legal limitations for transgenic strains, we conclude that the development of genetic sexing strains through classical genetic methods likely represents the best option for SIT-based operational programs.

Acknowledgments

We thank K. Bourtzis for encouraging us in this literature review, and T. Williams for English editing. BMMA (CVU: 885264) thanks the Consejo Nacional de Ciencia y Tecnología (CONACyT, Mexico) for providing a Master's scholarship, and the FAO-IAEA Joint Division TC project MEX5032 for providing a training fellowship at the Insect Pest Control Laboratories at Seibersdorf, Austria.

References

Alphey L, Benedict M, Bellini R, Clark GG, Dame DA et al. (2010) Sterile-insect methods for control of mosquito-borne diseases: an analysis. Vector-Borne and Zoonotic Diseases 10: 295–311.

Araújo HR, Carvalho DO, Ioshino RS, Costa-da-Silva AL & Capurro ML (2015) *Aedes aegypti* control strategies in Brazil: incorporation of new technologies to overcome the persistence of dengue epidemics. Insects 6: 576–594.

Arjona A, Wang P, Montgomery RR & Fikrig E (2011) Innate immune control of West Nile virus infection. Cellular Microbiology 13: 1648–1658.

Arredondo-García JL, Méndez-Herrera A & Medina-Cortina H (2016) Arbovirus in Latin America. Acta Pediátrica de México 37: 111–131.

Bailey DL, Lowe RE, Dame DA & Seawright JA (1980) Mass rearing the genetically altered MACHO strain of *Anopheles albimanus* Wiedemann. American Journal of Tropical Medicine and Hygiene 29: 141–149.

Baldacchino F, Caputo B, Chandre F, Drago A, della Torre A et al. (2015) Control methods against invasive *Aedes* mosquitoes in Europe: a review. Pest Management Science 71: 1471–1485.

Benedict M, D'Abbs P, Dobson S, Gottlieb M, Harrington L et al. (2008) Guidance for contained field trials of vector mosquitoes engineered to contain a gene drive system: recommendations of a scientific working group. Vector-Borne and Zoonotic Diseases 8: 127–166.

Benedict MQ & Robinson AS (2003) The first releases of transgenic mosquitoes: an argument for the sterile insect technique. Trends in Parasitology 19: 349–355.

Bourtzis K & O'Neill SL (1998) *Wolbachia* infections and arthropod reproduction. BioScience 48: 287–293.

Bourtzis K & Tu ZJ (2018) Joint FAO/IAEA coordinated research project on 'Exploring genetic, molecular, mechanical and behavioural methods of sex separation in mosquitoes' – an introduction. Parasites & Vectors 11: 653.

Breeland SG (1974) Population patterns of *Anopheles albimanus* and their significance to malaria abatement. Bulletin of the World Health Organization (Supplement) 50: 307–315.

Breeland SG, Boston MD, Kaiser R, Lofgren CS, Weidhaas DE et al. (1974) Release of chemosterilized males for the control of *Anopheles albimanus* in El Salvador. American Journal of Tropical Medicine and Hygiene 23: 288–297.

Breeland SG, Jeffery GM, Lofgren CS & Weidhaas DE (1974) Release of chemosterilized males for the control of *Anopheles albimanus* in El Salvador. American Journal of Tropical Medicine and Hygiene 23: 274–281.

Breeuwer JAJ, Stouthamer R, Burns DA, Pelletier DA, Weisburg WG & Werren JH (1992) Phylogeny of cytoplasmic incompatibility microorganisms in the parasitoid wasp genus *Nasonia* (Hymenoptera: Pteromalidae) based on 16s ribosomal DNA sequences. Insect Molecular Biology 1: 25–36.

Brelsfoard CL, St Clair W & Dobson SL (2009) Integration of irradiation with cytoplasmic incompatibility to facilitate a lymphatic filariasis vector elimination approach. Parasites & Vectors 2: 38.

Brownstein JS, Hett E & O'Neill SL (2003) The potential of virulent *Wolbachia* to modulate disease transmission by insects. Journal of Invertebrate Pathology 84: 24–29.

Capurro ML (2018) Regional efforts for combating zika virus in Latin America and the Caribbean. IAEA Technical Cooperation Programme: Sixty Years and Beyond – Contributing to Development. Proceedings of an International Conference 36: 49. IAEA, Vienna, Austria. (https://www-pub.iaea.org/books/iaeabooks/Supplementary_Materials/files/12280/100000/IAEA-Technical-Cooperation-Programme-Sixty-Years-Beyond-Contributing-Development, accessed 19 March 2020).

CDC (Centers for Disease Control and Prevention) (2010) http://www.cdc.gov/dengue/entomologyEcology. CDC, US Department of Health and Human Services, Atlanta, GA, USA (accessed 7 March 2020).

Craig GB Jr & Hickey WA (1967a) Genetics of *Aedes aegypti*. Genetics of Insect Vectors of Disease (eds. by JW Wright & R Pal), pp. 67–131. Elsevier, Amsterdam, The Netherlands.

Craig GB Jr & Hickey WA (1967b) Current status of the formal genetics of *Aedes aegypti*. Bulletin of the World Health Organization 36: 559–562.

Crawford JE, Clarke DW, Criswell V, Desnoyer M, Cornel D et al. (2020) Efficient production of male *Wolbachia*-infected *Aedes aegypti* mosquitoes enables large-scale suppression of wild populations. Nature Biotechnology 38: 482–492.

Curtis Cf, Brooks Gd, Ansari Ma, Grover Kk, Krishnamurthy Bs et al. (1982) A field trial on control of *Culex quinquefasciatus* by release of males of a strain integrating cytoplasmic incompatibility and a translocation. Entomologia Experimentalis et Applicata 31: 181–190.

Curtis CF & Graves PM (1988) Methods for replacement of malaria vector populations. American Journal of Tropical Medicine and Hygiene 91: 43–48.

Davidson G, Odetoyinbo JA, Colussa B & Coz J (1970) A field attempt to assess the mating competitiveness of sterile males produced by crossing 2 members of the *Anopheles gambiae* complex. Bulletin of the World Health Organization 42: 55–67.

Delatte H, Dehecq JS, Thiria J, Domerg C, Paupy C & Fontenille D (2008) Geographic distribution and developmental sites of *Aedes albopictus* (Diptera: Culicidae) during a chikungunya epidemic event. Vector-Borne and Zoonotic Diseases 8: 25–34.

Deredec A, Godfray HCJ & Burt A (2011) Requirements for effective malaria control with homing endonuclease genes.

Proceedings of the National Academy of Sciences of the USA 108: E874–E880.

DIR-SIT (World-Wide Directory of SIT facilities) (2020) http://nucleus.iaea.org/dirsit/DIRSITx.aspx (accessed 30 April 2021).

Dobson SL, Bourtzis K, Braig HR, Jones BF, Zhou W et al. (1999) *Wolbachia* infections are distributed throughout insect somatic and germ line tissues. Insect Biochemistry and Molecular Biology 2: 153–160.

Dyck VA, Hendrichs J & Robinson AS (2021) Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management, 2nd edn. CRC Press, Boca Raton, FL, USA.

Enserink M (2010) GM mosquito trial alarms opponents, strains ties in Gates-funded project. Science 330: 1030–1031.

Facchinelli L, Valerio L, Ramsey JM & Gould F (2013) Field cage studies and progressive evaluation of genetically-engineered mosquitoes. PLoS Neglected Tropical Diseases 7: e2001.

Failloux AB, Vazeille M & Rodhain F (2002) Geographic genetic variation in populations of the dengue virus vector *Aedes aegypti*. Journal of Molecular Evolution 5: 653–663.

Favia G (2015) Engineered mosquitoes to fight mosquito borne diseases: not a merely technical issue. Bioengineered 6: 5–7.

Fay RM & Morlan HB (1959) A mechanical device for separating the developmental stages, sexes and species of mosquitoes. Mosquito News 19: 144–147.

Fernández-Salas I, Danis-Lozano R, Casas-Martínez M, Ulloa A, Bond JG et al. (2015) Historical inability to control *Aedes aegypti* as a main contributor of fast dispersal of chikungunya outbreaks in Latin America. Antiviral Research 124: 30–42.

Fialho RF & Stevens L (2000) Male-killing *Wolbachia* in a flour beetle. Proceedings of the Royal Society of London B 267: 1469–1473.

Franz G (2005) Genetic sexing strains in Mediterranean fruit fly, an example for other species amenable to large-scale rearing as required for the sterile insect technique: Sterile Insect Technique. Principles and Practice in Area-Wide Integrated Pest Management (eds. by VA Dyck, J Hendrichs & AS Robinson), pp. 427–451. Springer, Dordrecht, The Netherlands.

Franz G, Kerremans P, Rendon P & Hendrichs J (1996) Development and application of genetic sexing systems for the Mediterranean fruit fly based on a temperature sensitive lethal. Fruitfly Pests: A World Assessment of their Biology and Management (eds. by B McPheron & G Steck), pp. 185–191. St. Lucie Press, Delray Beach, FL, USA.

Gilles JRL, Schetelig MF, Scolari F, Marec F, Capurro ML et al. (2014) Towards mosquito sterile insect technique programmes: exploring genetic, molecular, mechanical and behavioural methods of sex separation in mosquitoes. Acta Tropica 132: S178–S187.

Grover KK, Curtis CF, Sharma VP, Singh KRP, Dietz K et al. (1976) Competitiveness of chemosterilised males and cytoplasmically incompatible translocated males of *Culex pipiens fatigans* Wiedemann (Diptera: Culicidae) in the field. Bulletin of Entomological Research 66: 469–480.

Gunathilaka N, Ranathunge T, Udayanga L, Wijegunawardena A, Gilles JRL & Abeyewickreme W (2019) Use of mechanical and behavioural methods to eliminate female *Aedes aegypti* and *Aedes albopictus* for sterile insect technique and incompatible insect technique applications. Parasites & Vectors 12: 148.

Handler AM (2002) Prospects for using genetic transformation for improved SIT and new biocontrol methods. Genetica 116: 137–149.

Harris AF, McKemey AR, Nimmo D, Curtis Z, Black I et al. (2012) Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. Nature Biotechnology 30: 828–830.

Harris AF, Nimmo D, McKemey AR, Kelly N, Scaife S et al. (2011) Field performance of engineered male mosquitoes. Nature Biotechnology 29: 1034–1037.

Hoffmann AA & Turelli M (1997) Cytoplasmic incompatibility in insects. Influential Passengers: Inherited Microorganisms and Arthropod Reproduction (eds. by SL O'Neill, AA Hoffmann & JH Werren), pp. 42–80. Oxford University Press, Oxford, UK.

Hurst GDD, Jiggins FM, von der Schulenburg JHG, Bertrand D, Stuart AW et al. (1999) Male-killing *Wolbachia* in two species of insect. Proceedings of the Royal Society B 266: 735–740.

Hurst GDD, Johnson AP, von der Schulenburg JHG & Fuyama Y (2000) Male-killing *Wolbachia* in *Drosophila*: a temperature sensitive trait with a threshold bacterial density. Genetics 156: 699–709.

Jiggins FM, Hurst GDD & Majerus MEN (2000) Sex-ratio-distorting *Wolbachia* causes sex-role reversal in its butterfly host. Proceedings of the Royal Society B 267: 69–73.

Juchault P, Frelon M, Bouchon D & Rigaud T (1994) New evidence for feminizing bacteria in terrestrial isopods: evolutionary implications. Comptes Rendus de l'Académie des Sciences III 317: 225–230.

Kittayapong P, Kaeothaisong NO, Ninphanomchai S & Limohpasmanee W (2018) Combined sterile insect technique and incompatible insect technique: sex separation and quality of sterile *Aedes aegypti* male mosquitoes released in a pilot population suppression trial in Thailand. Parasites & Vectors 11: 657.

Klassen W, Curtis CF & Hendrichs J (2021) History of the Sterile Insect Technique. Sterile Insect Technique: Principles and Practices in Area-wide Integrated Pest Management, 2nd edn (eds. by VA Dyck, J Hendrichs & AS Robinson), pp. 1–44. CRC Press, Boca Raton, FL, USA.

Knipling EF, Laven H, Craig GB, Pal R, Kitzmiller JB et al. (1968) Genetic control of insects of public health importance. Bulletin of the World Health Organization 38: 421.

Lacroix R, McKemey AR, Raduan N, Kwee Wee L, Hong Ming W et al. (2012) Open field release of genetically engineered sterile male *Aedes aegypti* in Malaysia. PLoS One 7: e42771.

Laven H (1967) Eradication of *Culex pipiens fatigans* through cytoplasmic incompatibility. Nature 216: 383–384.

Laven H (1971) Une expérience de lutte génétique contre *Culex pipiens fatigans* Wied., 1828. Annales de Parasitologie Humaine et Comparée 46: 117–148.

Laven H & Aslamkhan, M (1970) Control of *Culex pipiens* and *C.p. fatigans* with integrated systems. Pakistan Journal of Science 22: 303–312.

Lavery JV, Harrington LC & Scott TW (2008) Ethical, social, and cultural considerations for site selection for research with genetically modified mosquitoes. American Journal of Tropical Medicine and Hygiene 79: 312–318.

Lebon C, Benlali A, Atyame C, Mavingui P & Tortosa P (2018) Construction of a genetic sexing strain for *Aedes albopictus*: a promising tool for the development of sterilizing insect control strategies targeting the tiger mosquito. Parasites & Vectors 11: 57–64.

Lee Hl, Vasan S, Ahmad NW, Idris I, Hanum N et al. (2013) Mating compatibility and competitiveness of transgenic and wild type *Aedes aegypti* (L.) under contained semi-field conditions. Transgenic Research 22: 47–57.

Lees RS, Gilles JR, Hendrichs J, Vreysen MJ & Bourtzis K (2015) Back to the future: the sterile insect technique against mosquito disease vectors. Current Opinion in Insect Science 10: 156–162.

Lees RS, Knols B, Bellini R, Benedict MQ, Bheecarry A et al. (2014) Review: improving our knowledge of male mosquito biology in relation to genetic control programmes. Acta Tropica 132: S2–S11.

Macer D (2005) Ethical, legal and social issues of genetically modifying insect vectors for public health. Insect Biochemistry and Molecular Biology 35: 649–660.

Madakacherry O, Lees RS & Gilles JRL (2014) *Aedes albopictus* (Skuse) males in laboratory and semi-field cages: release ratios and mating competitiveness. Acta Tropica 132: S124–S129.

Mains JW, Brelsfoard CL, Rose RI & Dobson SL (2016) Female adult *Aedes albopictus* suppression by *Wolbachia*-infected male mosquitoes. Scientific Reports 6: 33846.

Malavasi A (2014) Project Aedes transgenic population control in Juazeiro and Jacobina Bahia, Brazil. BMC Proceedings 8: O11.

Marec F (1991) Genetic control of pest Lepidoptera: construction of a balanced lethal strain in *Ephestia kuehniella*. Entomologia Experimentalis et Applicata 61: 271–283.

Mateos M, Martinez Montoya H, Lanzavecchia SB, Conte C, Guillén K et al. (2020) *Wolbachia pipientis* associated with tephritid fruit fly pests: from basic research to applications. Frontiers in Microbiology 11: 1080.

McCombs SD & Saul SH (1992) Flightless mutants in the melon fly and oriental fruit fly (Diptera: Tephritidae) and their possible role in the sterile insect release method. Annals of the Entomological Society of America 85: 344–347.

McDonald PT, Hausermann W & Lorimer N (1977) Sterility introduced by release of genetically altered males to a domestic population of *Aedes aegypti* at the Kenya coast. American Journal of Tropical Medicine and Hygiene 26: 553–561.

McGraw EA & O'Neill SL (2013) Beyond insecticides: new thinking on an ancient problem. Nature Reviews Microbiology 11: 181–193.

McMeniman CJ, Lane RV, Cass BN, Fong AW, Sidhu M et al. (2009) Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. Science 323: 141–144.

Meza JS (2020) Desarrollo y uso de cepas transgénicas para la técnica del insecto estéril. Moscas de la Fruta: Fundamentos y Procedimientos para su. Manejo (eds. by P Montoya, J Toledo & E Hernández), pp. 573–586. S y G editors, Mexico City, Mexico.

Monath TP (2013) Vaccines against diseases transmitted from animals to humans: a one health paradigm. Vaccine 31: 5321–5338.

Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT et al. (2009) A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and plasmodium. Cell 139: 1268–1278.

Munhenga G, Brooke BD, Gilles JRL, Slabbert K, Kemp A et al. (2016) Mating competitiveness of sterile genetic sexing strain males (GAMA) under laboratory and semi-field conditions: steps towards the use of the sterile insect technique to control the major malaria vector *Anopheles arabiensis* in South Africa. Parasites & Vectors 9: 122.

Onchuru T, Ajamma Y, Burugu M, Kaltenpoth M, Masiga D & Villinger J (2016) Chemical parameters and bacterial communities associated with larval habitats of *Anopheles*, *Culex* and *Aedes* mosquitoes (Diptera: Culicidae) in Western Kenya. International Journal of Tropical Insect Science 36: 146–160.

O'Neill SL, Giordano R, Colbert AM, Karr TL & Robertson HM (1992) 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. Proceedings of the National Academy of Sciences of the USA 89: 2699–2702.

Papathanos PA, Bossin HC, Benedict MQ, Catteruccia F, Malcolm CA et al. (2009) Sex separation strategies: past experience and new approaches. Malaria Journal 8: S5.

Papathanos PA, Bourtzis K, Tripet F, Bossin H, Virginio JF et al. (2018) A perspective on the need and current status of efficient sex separation methods for mosquito genetic control. Parasites & Vectors 11: 165–171.

Patterson RS, Weidhaas DE, Ford HR & Lofgren CS (1970) Suppression and elimination of an island population of *Culex pipiens quinquefasciatus* with sterile males. Science 168: 1368–1370.

Paupy C, Ollomo B, Kamgang B, Moutailler S, Rousset D et al. (2010) Comparative role of *Aedes albopictus* and *Aedes aegypti* in the emergence of dengue and chikungunya in central Africa. Vector-Borne and Zoonotic Diseases 10: 259–266.

Petersen JL, Lounibos LP & Lorimer N (1977) Field trials of double translocation heterozygote males for genetic control of *Aedes aegypti* (L.) (Diptera: Culicidae). Bulletin of Entomological Research 67: 313–324.

Phuc H, Andreasen MH, Burton RS, Vass C, Epton MJ et al. (2007) Late-acting dominant lethal genetic systems and mosquito control. BMC Biology 5: 11.

Quemada H (2016) Regulation of transgenic mosquitoes. Genetic Control of Malaria and Dengue (ed. by ZN Adelman), pp. 363–373. Academic Press, Blacksburg, VA, USA.

Ramsey JM, Bond JG, Macotela ME, Facchinelli L, Valerio L et al. (2014) A regulatory structure for working with genetically modified mosquitoes: lessons from Mexico. PLoS Negleted Tropical Diseases 8: e2623.

Reeves RG, Denton JA, Santucci F, Bryk J & Reed FA (2012) Scientific standards and the regulation of genetically modified insects. PLoS Neglected Tropical Diseases 6: e1502.

Reiter P, Fontenille D & Paupy C (2006) *Aedes albopictus* as an epidemic vector of chikungunya virus: another emerging problem? Lancet Infectious Diseases 6: 463–464.

Rigaud T, Souty-Grosset C, Raimond R, Mocquard JP & Juchault P (1991) Feminizing endocytobiosis in the terrestrial crustacean *Armadillidium vulgare* Latr. (Isopoda): recent acquisitions. Endocytobiosis and Cell Research 7: 259–273.

Robinson A (2002) Mutations and their use in insect control. Mutation Research/Reviews in Mutation Research 511: 113–132.

Rössler Y (1979) Automated sexing of *Ceratitis capitata* (Diptera: Tephritidae): the development of strains with inherited, sex-limited pupal color dimorphism. Entomophaga 24: 411–416.

Rössler Y & Rosenthal H (1988) Genetics of the Mediterranean fruit fly (Diptera: Tephritidae): eye color, eye shape and wing mutations. Annals of the Entomological Society of America 81: 350–355.

Rousset F, Bouchon D, Pintureau B, Juchault P & Solignac M (1992) *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. Proceedings of the Royal Society B 250: 91–98.

Seawright JA, Kasier PE, Dame DA & Lofgren CS (1978) Genetic method for the preferential elimination of females of *Anopheles albimanus*. Science 200: 1303–1304.

Shragai T, Tesla B, Murdock C & Harrington LC (2017) Zika and chikungunya: mosquito-borne viruses in a changing world: global change and vectors of chikungunya and Zika. Annals of the New York Academy of Sciences 1399: 61–77.

Skerritt JH (2000) Genetically modified plants: developing countries and the public acceptance debate. AgBiotechNet 2(ABN 040): 1–4.

Stouthamer R, Breeuwer JAJ, Luck RF & Werren JH (1993) Molecular identification of microorganisms associated with parthenogenesis. Nature 361: 66–68.

Subbaraman N (2011) Science snipes at Oxitec transgenic-mosquito trial. Nature Biotechnology 29: 1.

Tandina F, Doumbo O, Yaro AS, Traoré SF, Parola P & Robert V (2018) Mosquitoes (Diptera: Culicidae) and mosquito-borne diseases in Mali, West Africa. Parasites & Vectors 11: 1–12.

Tazima Y, Harada C & Ohata N (1951) On the sex discriminating method by coloring genes of silkworm eggs. Part I. Induction of translocation between the W and the 10th chromosomes. Japanese Journal of Breeding 1: 47–50.

Terenius O, Marinotti O, Sieglaff D & James AA (2008) Molecular genetic manipulation of vector mosquitoes. Cell Host & Microbe 4: 417–423.

Traver BE, Anderson MA & Adelman ZN (2009) Homing endonucleases catalyze double-stranded DNA breaks and somatic transgene excision in *Aedes aegypti*. Insect Molecular Biology 18: 623–633.

Wade MJ & Stevens L (1985) Microorganism mediated reproductive isolation in flour beetles (genus *Tribolium*). Science 227: 527–528.

Weidhaas DE, Breeland SG, Lofgren CS, Dame DA & Kaiser R (1974) Release of chemosterilized males for the control of *Anopheles albimanus* in El Salvador. IV. Dynamics of the test population. American Journal of Tropical Medicine and Hygiene 23: 298–308.

Weidhaas DE, Schmidt CH & Seabrook EL (1962) Field studies on the release of sterile males for the control of *Anopheles quadrimaculatus*. Mosquito News 22: 283–291.

Werren JH, Zhang W & Guo R (1995) Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. Proceedings of the Royal Society B 261: 55–63.

WHO (World Health Organization) (1964) Genetics of Vectors and Insecticide Resistance: Report of a WHO Scientific Group [meeting held in Geneva from 5 to 9 August 1963]. WHO, Geneva, Switzerland.

WHO (World Health Organization) (2003) Space Spray Application of Insecticides for Vector and Public Health Pest Control: A Practitioner's Guide. Communicable Disease Control, Prevention and Eradication WHO Pesticide Evaluation Scheme (WHOPES), WHO Geneva, Switzerland. (http://whqlibdoc.who.int/hq/2003/WHO_CDS_WHOPES_GCDPP_2003.5.pdf, accessed 16 May 2020).

WHO/EMRO (World Health Organization, Regional Office for the Eastern Mediterranean) (2003) Use of Fish for Mosquito Control. WHO/EMRO, Cairo, Egypt.

WHO/IAEA (World Health Organization/International Atomic Energy Agency) (2020) Guidance Framework for Testing the Sterile Insect Technique as a Vector Control Tool Against Aedes-borne Diseases. WHO, Geneva, Switzerland. (http://www.who.int/tdr/publications/year/2020/guidance-framework-for-testing-SIT/en/ (accessed 27 June 2020).

Wilke ABB, Gomes ADC, Natal D & Marrelli MT (2009) Control of vector populations using genetically modified mosquitoes. Revista de Saude Publica 43: 869–874.

Windbichler N, Menichelli M, Papathanos PA, Thyme SB, Li H et al. (2011) A synthetic homing endonuclease-based gene drive system in the human malaria mosquito. Nature 473: 212–215.

Yamada H, Benedict MQ, Malcolm CA, Oliva CF, Soliban SM & Gilles JRL (2012) Genetic sex separation of the malaria vector, *Anopheles arabiensis*, by exposing eggs to dieldrin. Malaria Journal 11: 208.

Zacarés M, Salvador-Herranz G, Almenar D, Tur C, Argilés R et al. (2018) Exploring the potential of computer vision analysis of pupae size dimorphism for adaptive sex sorting systems of various vector mosquito species. Parasites & Vectors 11: 656.

Zeh DW, Zeh JA & Bonilla MM (2005) *Wolbachia*, sex ratio bias and apparent male killing in the harlequin beetle riding pseudoscorpion. Heredity 95: 41–49.

Zheng X, Zhang D, Li Y, Yang C, Wu Y et al. (2019) Incompatible and sterile insect techniques combined eliminate mosquitoes. Nature 572: 56–61.