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Back to the future: the sterile insect technique against mosquito disease vectors

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With the global burden of mosquito-borne diseases increasing, and some conventional vector control tools losing effectiveness, the sterile insect technique (SIT) is a potential new tool in the arsenal. Equipment and protocols have been developed and validated for efficient mass-rearing, irradiation and release of Aedines and Anophelines that could be useful for several control approaches. Assessment of male quality is becoming more sophisticated, and several groups are well advanced in pilot site selection and population surveillance. It will not be long before SIT feasibility has been evaluated in various settings. Until perfect sexing mechanisms exist, combination of *Wolbachia*-induced phenotypes, such as cytoplasmic incompatibility and pathogen interference, and irradiation may prove to be the safest solution for population suppression.

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Introduction

The many pathogens transmitted by mosquitoes (Diptera: Culicidae) which feed on the blood of humans in order to mature their eggs are responsible for enormous suffering worldwide. Annual deaths from malaria alone number at least 600,000, up to 100,000 people contract dengue each year, and Chikungunya causes severe chronic joint pain in patients across the globe (World Health Organization factsheet; URL: http://www.who.int/mediacentre/factsheets/fs387/en/). Aside from causing mortality and morbidity, the economic and social burden from these diseases is significant [65], particularly in SubSaharan Africa (Multisectoral Action Framework for Malaria; URL: http://reliefweb.int/sites/reliefweb.int/files/resources/Multisectoral-Action-Framework-for-Malaria.pdf).

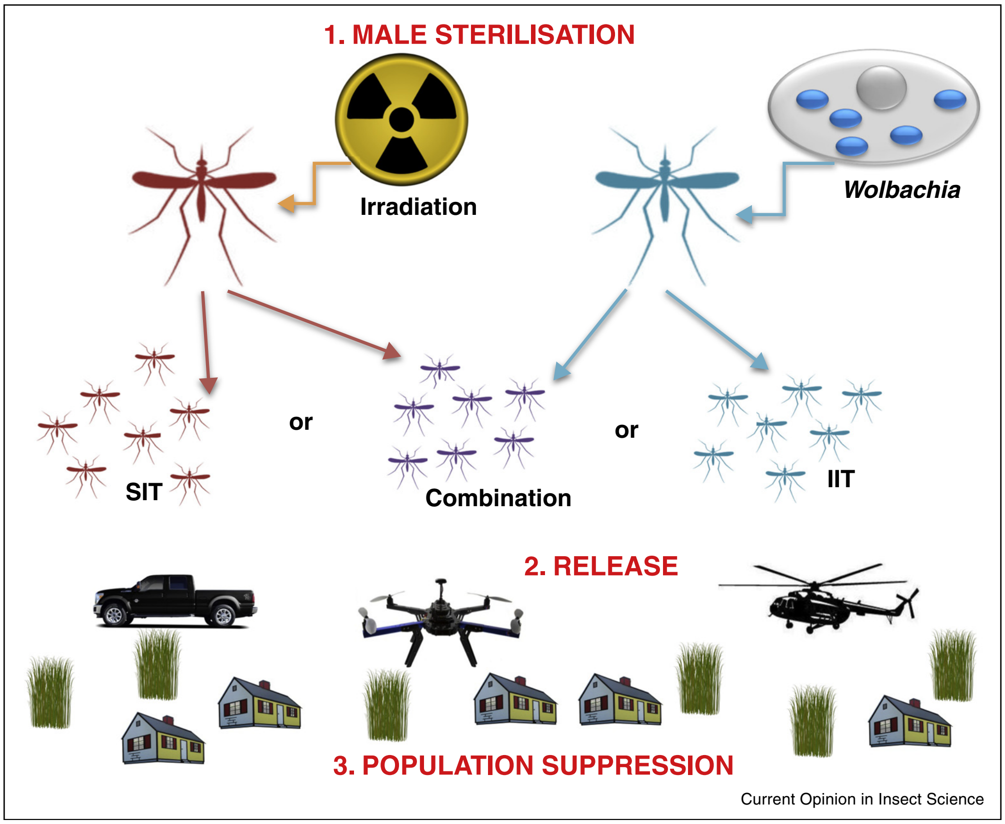
The pressure placed on humanity by these vectors is increasing. Expansion of the distribution of several invasive *Aedes* species such as *Aedes albopictus* [1] is evident in many areas, including Europe [2,3] and USA [4]. Modelling and field experiments have predicted that *Ae. albopictus* has the potential to invade large areas of Australia [5] and urbanisation is increasing its abundance in China [6]. With no effective vaccines or specific drugs to prevent or treat mosquito-borne diseases, the best line of defence is to combat the vector, to remove the contact between mosquitoes and humans and thus interrupt the disease transmission cycle. Effective mosquito control is hindered by growing insecticide resistance of malaria [7] and dengue vectors [8], even in regions only recently invaded (e.g. [9]). There is therefore increasing demand for complementary tactics that are effective, more sustainable and friendly to the environment.

One such tactic could be the sterile insect technique (SIT), which relies on the production and release of sufficient sterile males to induce sterility in the wild females which, over time, causes the target population to decline (Figure 1). The SIT has no regulatory requirements and the technique would be combined with others as part of an area-wide integrated pest management (AW-IPM) approach to reduce the vector population below the threshold required for disease transmission. Sterilisation using ionising radiation has been extremely effective and applied successfully for population suppression, containment or eradication of several major pest insect species [10].

Rather than sterilising males using irradiation, an alternative method is to exploit the natural phenomenon of cytoplasmic incompatibility (CI). In most diplodiploid species, CI is expressed as embryonic lethality after matings between *Wolbachia*-infected males and uninfected females or females infected with a different *Wolbachia* strain [11]. Proof-of-concept has been provided that CI could be used to manage agricultural pests and disease vectors through population suppression or replacement approaches [12•,13,14]. CI-based population suppression is known as the incompatible insect technique (IIT) (Figure 1).

As the key mosquito disease vectors are all relatively amenable to colonisation and rearing, and in many

Figure 1



The sterile insect technique (SIT), incompatible insect technique (IIT) or a combination of the two could be used to suppress mosquito populations. Male mosquitoes are sterilised either by the application of irradiation or (trans)infection with *Wolbachia*, or both, and then released into the target population to sterilise the wild females.

situations the natural population densities are low, the SIT, IIT, or a combination of the two are well suited for their management. The advantages of combining these tactics will be discussed in this review, alongside the current state of the art for the two approaches. Much progress has been made in recent years towards developing the required technology and methodology to bring mosquito suppression using sterility to field application; indeed pilot releases have begun in a number of sites around the world. It should be mentioned, however, that a number of other technologies have also been developed and are being tested in pilot trials including RIDL (Release of Insects carrying a Dominant Lethal) and *Wolbachia*-based population replacement strategies. However, it is beyond the scope of this review to discuss these approaches, and they have recently been reviewed elsewhere [13,15].

Developing the sterile insect technique against mosquitoes

After a period of enthusiasm in the 1960s to early 1980s [16], the use of sterile male release for mosquito control was largely abandoned. However, the growing pressures from mosquito-borne pathogens described above, and the proposed use of modern biotechnologies to sterilise or otherwise alter mosquitoes, have led to revived interest in recent years.

In the last decade, the Joint FAO/IAEA Programme and their collaborators have been the main drivers for the development of the "SIT package" for mosquitoes. Requests from many countries to develop and evaluate the SIT for use against mosquitoes have spurred the development and ongoing validation of mass-rearing equipment, diet and protocols for *Anopheles* and *Aedes* species. Diets have been optimised to feed the larval stages of *An. gambiae* [17], *An. arabiensis* [18], *An. stephensi* [19] and *Ae. albopictus* [20]. *Anopheles* larvae can be mass-reared efficiently in large trays fitted into a novel tilting rack system [21,22], and the system is being validated for *Aedes* species. Anopheline pupae can be separated from larvae based on differential buoyancy using custom vortex equipment [23], or the Fay-Morlan separator used for *Aedes* [24], quantified volumetrically, and allowed to emerge into adult mass-rearing cages [25]. Blood meals are offered to females using a modified hemotec membrane feeder and water is added to the cage floor for oviposition [26]. Eggs are flushed from these cages and can be quantified ([27] for *Aedes*, Maiga *et al.*, personal communication for *Anopheles*), stored and hatched effectively [28] to give a reliable quantity of eggs and so a predictable larval density in rearing trays.

A method to accurately separate males from females on a large scale, crucial for the required male-only release, is still required [29], particularly for *Anopheles*, which do not have the sexual dimorphism that allows the sexing of *Aedes* on a small-medium scale [24,30•]. A method to spike blood meals with Ivermectin [31] is a reasonable stop-gap solution. This requirement for sex separation applies for all mosquito suppression methods based on release, including those described below. The methods for radiation–sterilisation of mosquitoes have long been available, developed alongside those for many other target species, but have more recently been revisited to optimise doses [32–34], and to assess the use of X-rays as an alternative for gamma irradiation [35,36].

Progress in SIT field application

The vanguard in reviving the use of the SIT against mosquitoes was an Italian group [37] who released around 1000 irradiated *Ae. albopictus* pupae per hectare per week, inducing up to 68% sterility in the target populations in three pilot sites of between 16 and 45 ha [38•]. Releases continued for 5 years, and demonstrated the potential of sterile males to suppress an *Ae. albopictus* population.

The importance of quality management of sterile mosquito males to ensure adequate performance and competitiveness after release is evident from examples in other species [39]; the estimation and quantification of the impact of mass-rearing, radiation and handling on male mating competitiveness of sterile males has attracted a lot of research. Semi-field and field experiments have demonstrated that a radiation dose can be selected that gives sufficient sterility without significantly impacting competitiveness [40,41,42•]. With this reassurance, several vector control groups, supported by the FAO/IAEA, are conducting preparatory activities and initiating pilot trials that include the SIT.

The first step in assessing the SIT in a given context is to select proper pilot sites, which should have a manageable size, a low mosquito population density and a good level of geographical or biological isolation, among other criteria reviewed in Malcolm *et al.* [43] and Brown *et al.* [44]. Two such sites have been identified by the Ministry of Health and Quality of Life in Mauritius [45], where they have also completed the second preparatory step, the long-term surveillance of the *Aedes albopictus* natural population using ovitraps and BG-Sentinels to trap adults. A project in La Réunion has progressed to a similar stage [46]. A good understanding of the biology, dynamics and distribution of the male population in the target area is crucial to properly plan the releases and to monitor their effect. Although several effective traps exist for male *Aedes* surveillance, male *Anopheles* are much more difficult to monitor.

In Sudan, the target species, *An. arabiensis*, is contained along a narrow strip on either side of the River Nile, and surveillance has demonstrated temporal variations of population densities that were overall low [47]. Further, mark-release-recapture experiments have demonstrated the ability of radiation-sterilised males to locate and participate in naturally occurring swarms, or to start new swarms [42•]. Encouraged by these data, small-scale releases have started, and construction of a mass-rearing facility is scheduled to supply the sterile mosquitoes for suppression trials. A project in South Africa, targeting *An. arabiensis*, is at a similar stage of advancement [48]. A coordinated research project (CRP) is being initiated by the FAO/IAEA (''Mosquito Handling, Transport, Release and Male Trapping Methods'') to support these projects in developing and validating suitable methods for releasing sterile male mosquitoes and surveying the target population before, during and after suppression trials (http://www-naweb.iaea.org/nafa/ipc/index.html).

With these pieces in place, the whole SIT package for mosquitoes is coming closer to full scale field trials, and it is expected that within a very few years multiple feasibility studies in a range of settings and against a number of species will have been completed and have demonstrated the effectiveness and applicability of the technique against these disease vectors.

Incompatible insect technique: an additional tool and its potential combination with the sterile insect technique for population suppression

About 50 years ago *Wolbachia*-induced CI as a tool to suppress natural populations of *Culex pipiens fatigans* was used for the first time [49]. During the last few years there have been significant developments, both in the laboratory and in the field, towards the use of IIT for population suppression of mosquito vectors. There are also self-sustaining *Wolbachia*-based approaches that target population replacement with CI-inducing and pathogen-blocking strains; however the applicability, effectiveness and sustainability of this strategy require more studies [13,50].

Wolbachia-infected lines of *Culex pipiens quinquefasciatus* were selected and tested in laboratory cages for CI expression and population suppression of four natural populations originating from four islands: Grand Glorieuse, Mauritius, Mayotte and La Réunion [51•,52•]. The results of these trials were very promising, indicating that

C. p. quinquefasciatus males infected with the *Wolbachia* strain *w*Pip(Is) were fully incompatible (100% CI) with females from the four islands of the south-western Indian Ocean [51•]. As a next step, semi-field experiments were run showing that the *w*Pip(Is) males were: (a) able to induce complete CI in La Réunion field females and (b) fully competitive against field-collected males to mate with field-collected females [52•]. Similar IIT-based pilot trails were implemented against *Aedes polynesiensis*, with a proof-of-concept pilot trial in the Society Islands [53,54], and an ongoing field trial in French Polynesia (Bossin, personal communication).

New *Wolbachia* infection and CI types have been developed for one of the major dengue vectors, *Aedes albopictus* [55]. One of the lines, ARwP, is infected with a *w*Pip strain which naturally occurs in *Culex pipiens*. Mating experiments have shown that ARwP males exhibited full CI with uninfected females or naturally double-infected females (*w*AlbA and *w*AlbB), suggesting that this strain could in principle be used for population suppression. The use of ARwP (*w*Pip) males to suppress naturally double-infected (*w*AlbA and *w*AlbB) *Ae. albopictus* populations would be advantageous if there was complete CI between these strains, to minimise the risk from any accidental release of *w*Pip-infected females. However a recent study showed that crosses between males with low *w*AlbA density and ARwP females were partially fertile [56]. This finding suggests that the accidental release of *w*Pip-infected ARwP females may jeopardise a population suppression programme by instead causing population replacement. Thus IIT application will require a fail-proof sexing method so that it could be used as a tactic to suppress populations of mosquito vector species in a way similar to the SIT.

The requirement for perfect separation of males and females prior to release, discussed above, is particularly important for IIT because the accidental release of females may result in the loss of IIT and render a population suppression programme into population replacement. A possible strategy to manage this risk is to combine SIT and IIT (Figure 1) [14,53,57,58]. A strategy combining a low radiation dose to ensure female sterility (SIT), and IIT is being initiated against *Ae. albopictus*. A triple-infected line (*w*AlbA, *w*AlbB and *w*Pip) was shown to be completely incompatible with double-infected (*w*AlbA and *w*AlbB) lines as well as providing protection against dengue (Xi, unpublished data). No significant negative impact of the triple infection on several fitness traits could be measured [59], though the time required for immature development was significantly reduced in males compared to females, a finding which could be explored for sex separation.

Until recently, *Anopheles* species were considered to be *Wolbachia*-free. However, *Wolbachia* was recently detected in a natural population of *Anopheles gambiae* in Burkina Faso [60]. The *w*AlbB strain was recently transferred from *Ae. albopictus* to *Anopheles stephensi* creating a new stable transinfected line expressing complete CI, produced for population replacement strategies, but potentially effective for population suppression [13]. Taken together, these data suggests that *Anopheles* species are not ''resistant'' to *Wolbachia* infection and that IIT could also be used for population suppression of Anophelines.

Conclusion

In response to the growing interest and demand for the development and application of the SIT, with possible combination with the related IIT against mosquito vectors, significant advances have been made in developing the required equipment and protocols for rearing, sterilising and assessing the quality of male *Aedes* and *Anopheles* mosquitoes. Most of the pieces are thus in place for the technique to be validated in suppression programmes on a small scale, and in several different settings the preliminary work of site selection, population surveillance, upscaling of rearing and quality control is well advanced.

Before large scale releases are feasible, however, more efficient and less labour intensive methods are needed for transporting and releasing male mosquitoes into the field, as well as more effective methods for monitoring programme progress, particularly for *Anopheles* species. These are fairly simple design and engineering questions, which some time and careful evaluation in the field will be able to address in due course. The other major challenge is the development of an accurate sex separation method which can be applied on a large scale, which may require more sophisticated developments [29]. Until perfect sexing is available, the combination of SIT and IIT could be used to ensure that any unintentionally released *Wolbachia*-infected females would be sterile. In addition, *Wolbachia* transinfection may provide protection against the establishment, replication and/or transmission of *Plasmodium*, also eliminating the risk of disease transmission [13,14,61]. Once these remaining pieces are in place, sterile male release programmes hold great promise for control of mosquito vectors, particularly in urban areas where the human population to be protected is concentrated.

The effects of any genetic manipulation on the robustness and competitiveness of male mosquitoes in an open field setting is difficult to know before release. It is likely that genetic modification will have an impact [62], though the extent of the effect will be strain-specific. *Wolbachia* transinfection may or may not negatively impact mosquitoes [59,63], and may not interrupt disease transmission. With the application of radiation it is possible to adapt the dose to induce an adequate level of sterility whilst minimising the effect on male performance. The random mutations and gross gonad damage caused by irradiation

[16] eliminate the risk of resistance, which is a major problem with insecticide use, and potentially with genetic control measures [64]. Finally, in circumstances where there is public or regulatory opposition to the use of genetically modified organisms, release of fertile *Wolbachia*-infected females for population replacement, or generic insecticides, the SIT and the SIT–IIT combination offer an acceptable alternative. It is hoped that the effectiveness of these techniques can be demonstrated in the near future, and if proved effective, another powerful tool will have been added to the limited arsenal available for use against mosquito-borne diseases.

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