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SI-MOLECULAR TECHNOLOGIES TO IMPROVE SIT

Developing transgenic *Anopheles* mosquitoes for the sterile insect technique

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Abstract In the last 10 years the availability of the genome sequence of *Anopheles gambiae* and the development of a transgenic technology for several species of *Anopheles* mosquitoes have, in combination, helped in enabling us to gain several insights into the biology of these mosquitoes that is relevant to their capacity as vectors of the malaria parasite. While this information is anticipated to inform many novel vector control strategies, the technique most likely to benefit in the near future from the availability of a reliable transgenic technology is the sterile insect technique (SIT), which relies on releasing large numbers of sterile insects to compete for mates in the wild, leading to population suppression. Although SIT has been proven to work reliably for many insects, the construction of suitable strains, and induction of sterility, has until now been a laborious process, combining classical genetics with radiation-induced sterility. Using transgenesis to create strains of *Anopheles* suitable for SIT could potentially offer several advantages over current approaches, in that the basic design of transgenic constructs designed for other insects should be rapidly transferable to mosquitoes, and induction of sterility as a product of the transgenic modification could obviate the requirement for radiation and its associated deleterious effects. In this paper the progress of different transgenic approaches in constructing tools for SIT will be reviewed.

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Introduction

Anopheles mosquitoes, the exclusive vectors of malaria, represent a global health problem that is concentrated largely in developing regions. Malaria control efforts in the past have focused on both the disease-causing *Plasmodium* parasite and the *Anopheles* mosquitoes that exclusively transmit the parasite between human hosts. However, anti-parasitic measures such as drug and vaccine development have been compromised by repeated selection of mutations conferring drug-resistance in parasite populations and by the failure to induce sufficient long-lasting protective immunity, respectively. Similarly, the efficiency of programs employing large scale spraying of insecticides has been compromised by the rapid spread of resistance throughout the targeted mosquito populations. Nonetheless, of all the control methods so far used against malaria those that target the vector have proved the most successful. In this light the sterile insect technique (SIT), currently used successfully against a wide range of insect pests, represents a promising approach. SIT involves the release of large numbers of sterile individuals to cause population suppression through unproductive matings with the native, wild population.

Requirements of an *Anopheles* strain suitable for SIT

There are several well-documented cases of the successful use of SIT including the elimination of the New World screwworm, a livestock pest, from both Libya and North America and in the drastic reduction of populations of Mediterranean

fruit fly, an economically important fruit pest, across large areas of Central America (Klassen and Curtis 2005).

The first obvious requirement for SIT is an ability to mass rear the insect of choice to a quality that will allow the population to compete for matings in the wild.

In the example of *Ceratitis capitata*, flies are routinely reared and released in the order of several billion per week (Franz 2005). Although the efficiency and rearing capacity of production will, to some extent, be species-specific, the high numbers achieved with *C. capitata* highlight the feasibility of rearing such numbers as will be required in order to sufficiently interfere with the reproductive potential of large native populations over wide areas in SIT programs against mosquitoes. Moreover, a small scale sterile release of *Anopheles albimanus* was successful in El Salvador and a later program in the same country was able to rear millions of mosquitoes per week (reviewed in (Benedict and Robinson 2003)).

In addition to the capacity to rear sufficient numbers of insects, the other obvious and essential requirement is a method to induce sterility in this population immediately prior to its release. There are several proposed mechanisms for inducing sterility including chemosterilization, cytoplasmic incompatibility (where males infected with the intracellular bacterium *Wolbachia* are sterile when mated to non-infected females) and hybrid sterility, where two closely related species are mated to produce sterile hybrids for release. However, by far the most commonly used method of sterilization in SIT programs to date has been exposure to gamma-radiation which causes chromosomal breaks in the germ cells, leading to breakdown in maintenance of genetic information after fertilization and death in the early developing embryo. In principle, sterilization through irradiation is applicable to any insect pest although the correct dose has to be empirically established in order to ensure sufficient germ cell damage to cause sterility yet at the same time minimising somatic damage that will reduce the overall fitness and mating competitiveness of the release population.

In addition to optimisation of rearing conditions and the induction of sterility, there are many other factors that together help increase the likelihood of a successful SIT program. These include: knowledge of the population genetics and dynamics of the target insect population; methods to monitor the efficiency of mating between the release population and the target population; a detailed understanding of the post-mating response induced in females after copulation with sterile males; an efficient method to separate the sexes such that only males are released- sterile males, rather than sterile females, are 'the active agent in SIT' (Franz 2005) and the concurrent release of both males and females is less efficient due to a high incidence of sterile males mating with co-released sterile females (Rendon et al. 2004). In the case of *Anopheles* mosquitoes, and many other disease vectors, the requirement for an efficient sexing mechanism is absolute since released sterile females will pose a biting nuisance and, more importantly, will still be able to transmit disease.

To resolve many of the above issues, the availability of the *Anopheles gambiae* genome sequence (Holt et al. 2002), coupled with a transgenic technology that could introduce sterility genes, sex-specific lethal genes or marker genes for population studies is likely to be of great help in augmenting the capacity and potential of SIT approaches to *Anopheles* control.

Improvements to SIT offered by transgenic technologies

In recent years a transgenic technology has been developed for the major malaria vector in Africa, *Anopheles gambiae* (Grossman et al. 2001), the Indian vector *A. stephensi* (Catteruccia et al. 2000), and the South American malaria vector, *A. albimanus* (Perera et al. 2002). Despite initially taking a long time to develop, germline transformation of these vectors is now almost routine and is performed in several laboratories. The technology is based on the germline insertion of non-autonomous transposons in the presence, *in trans*, of the transposase enzyme that catalyzes the insertion but is itself unable to integrate. Thus the inserted transposons, containing a gene of interest, are stably integrated. Indeed, recent studies have shown that it is difficult to re-mobilise such elements even in the presence of an exogenous source of transposase.

The robustness of this technology means that there are several specific areas of the SIT approach that could directly benefit. In particular these are given below.

Strategies for transgenic sexing

Given the need to release only males in most forms of SIT for vectors of disease, a reliable method is required to separate the two sexes prior to release. For mosquitoes such as *Anopheles*, there exist some naturally occurring sexually dimorphic features that could, in principle, allow such a separation. For example, differences in pupal size in anophelines, where males being on average slightly smaller than females has allowed mechanical separation, although this has only ever achieved about 90% efficiency of separation (Dame et al. 1974). Even if 100% efficiency were achievable, there remain potential issues with physical damage caused by the mechanical separation (Calkins and Parker 2005) or the potential effects on the competitiveness of these males resulting from selection for constantly smaller males. As an alternative, other approaches have relied on making genetic sexing strains (GSS) that allow

separation of the sexes as a feature of their genetic composition. In many cases it has been possible to translocate naturally arising selectable markers to the sex chromosomes. To control the Mediterranean fruit fly *C. capitata* there are currently several area-wide SIT programs that use one GSS (Vienna 8) containing a Y chromosome-based translocation that rescues a temperature sensitive lethal mutation (Franz 2005). There are several examples where transfer of this technology to mosquitoes has been possible, with the generation of translocation strains, in which the locus conferring resistance to the insecticide was translocated to the Y chromosome, allowing for efficient female elimination (Curtis 1978, 1979; Curtis et al. 1976; Kaiser et al. 1978). However, there are costs and risks associated with such an approach: (a) the mutagen used to induce the translocation can cause fitness reduction due to collateral damage other than the translocated region; (b) the linkage of the translocated selectable marker and the sex chromosome can breakdown due to recombination (Franz 2002); (c) semi-sterility associated with translocations (due to chromosomal deletions and duplications during meiosis as a direct result of the translocation); (d) in the absence of complete sterility insecticide resistance alleles would introgress into the wild population. Obviously, the generation of such strains first requires the occurrence of a mutation conferring a selectable trait in each species of interest, followed by the serendipitous and lengthy process of screening for its translocation to the sex chromosome. In light of this, a transgenic approach to creating GSSs has the advantage that, often, the basic principles of the transgenic construct will be the same for a wide range of insect species.

Transgenic sexing by male selection

Genes encoding fluorescent proteins such as EGFP and RFP have been used successfully as transgenic markers from mammals to plants to insects. The expression of these genes under the control of sex-specific promoters or when inserted on to the sex chromosomes can allow sex-separation based on fluorescence. Such a system was initially developed in *A. stephensi* using the control regions of the sperm-specific β2-tubulin gene from *A. gambiae* to drive EGFP expression (Catteruccia et al. 2005). The sperm of transgenic individuals were EGFP-positive and males could be identified due to the presence of fluorescent testes as early as the 3rd instar larval stage. Highlighting the transferability of the transgenic technology, the same β2-tubulin::EGFP constructs have been shown to give an identical phenotype in *A. gambiae* (Windbichler et al. 2008) and *A. arabiensis* (J. Thailayil pers. comm.). Reliable separation based on this phenotype was shown in *A. stephensi* using the COPAS mechano-optical sorting system (Catteruccia et al. 2005). However, while this shows proof of principle for the technology, vast improvements in the efficiency and cost of the mechano-optical sorting would need to be achieved in order for it to become feasible on a the scale needed for an SIT program. A further advantage of using sperm-specific visible marker genes in *Anopheles* is that, after mating, sperm are stored for long periods in the spermatheca of the female, a feature that could be used to measure the frequency of matings between females in the wild and the transgenic released males.

More recently, the *Anopheles gambiae* orthologue of the *Drosophila melanogaste*r germline-specific *vasa* gene was identified and its control regions used to direct EGFP expression (Papathanos et al. 2009). Interestingly, two different constructs containing different lengths of sequence upstream of the start codon gave different expression patterns; one construct was entirely male-specific, limited to the testes and already visible in neonatal larvae while the other was similarly germline-restricted but expressed in both sexes. The male-specific construct is of particular use in the creation of a GSS, given that it would allow sexing to be performed at a very early developmental stage and minimising the wastage of resources in raising unwanted female larvae. The unexpected observation of differential sex-restriction between the two *vasa*-based constructs highlights the fact that engineering a GSS will not always be as straightforward as taking arbitrary sections of the upstream and downstream regulatory regions of candidate genes and using these to drive expression of the desired effector gene. In addition to sex-specific promoters it is also possible to take advantage of sex-specific alternative splicing in order to efficiently sex insect populations. The basic principle of this strategy is to engineer a selectable marker gene to contain sex-specifically spliced introns, in such a way that a functional coding sequence is only created as a result of correct splicing in the appropriate sex. Many genes in the sex determination pathway, such as *transformer* and *doublesex* are spliced alternatively between the sexes, and this is a feature that is conserved across a wide range of insects (Saccone et al. 2002; Telonis-Scott et al. 2009). In the Mediterranean fruit fly such a system, based on the incorporation of sex-specific introns from the *transformer* gene, has been shown to work (Fu et al. 2007). However, a similar approach in *A. gambiae*, based on a transgenic construct containing the EGFP gene inside the female-specific exon of *A. gambiae doublesex*, gave a surprising phenotype in that, rather than the expected female-specific EGFP expression, expression was seen in both sexes and was male-biased (Magnusson et al., manuscript submitted). The reasons for this observation are unclear but could be related to the genomic location of the transgene insertion in this single line or could be due to cryptic splice sites within the transgenic construct, both of which could interfere with the predicted splicing pattern. Alternatively, there might be

other, non sex-specific, patterns of *doublesex* splicing which render the approach unsuitable. Highlighting the difficulty of the method, attempts to construct a similar system in *D. melanogaster* by incorporation of sex-specifically spliced introns from the endogenous *doublesex* gene also failed (Scott et al. 2004). It is possible that other sex-specifically spliced members of the sex-determination pathway may lend themselves more to these types of sex-specifically spliced constructs. In this respect, efforts aimed at fully understanding the repertoire and complexity of genes involved in this pathway should be rewarding.

Transgenic sexing by female elimination

An alternative use of the transgenic approach in sexing, rather than selecting for males based on male-specific expression of positive selection traits, such as fluorescent proteins or insecticide resistance genes, could be to select against females by expressing lethal genes in a female-specific manner. This is the premise behind modifications of SIT such as Release of Insects carrying a Dominant Lethal (RIDL) (Alphey 2002; Thomas et al. 2000). Obviously, in order to rear an insect colony on a large scale, the lethal gene needs to be conditionally regulated such that it is expressed only in the final release generation. The conditional expression system most widely used in insects for this purpose is the tetracycline –repressible expression system ('Tet Off') (Gossen and Bujard 1992). Tet Off is a two-component system consisting of a tetracycline-repressible transactivator (tTA) driven by a promoter of choice and an effector gene under the transcriptional control of the tetO sequence, to which the tTA binds, leading to transcription of the gene of interest. Tetracycline provided in the factory rearing conditions ensures that the lethal effector gene is not expressed by interfering with the interaction of tTA with the tetO. In the absence of tetracycline (in the final rearing generation and in the wild) the gene is expressed in a pattern that is dependent on the transcriptional control elements that drive expression of the tTA. A female-specific conditional lethal system based on this system has been shown to work in the Mediterranean fruit fly (Fu et al. 2007), whilst components of the tetracycline-repressible system have been shown to work across a wide range of species including, importantly, *A. stephensi* (Lycett et al. 2004) meaning that the success of this system in *Anopheles* species is likely to depend more on the ability to engineer efficient sex-limited lethality.

Strategies for transgenic sterility

While transgenes that confer sterility or lethality phenotypes are of great potential advantage to SIT, such genes pose obvious complications in rearing sufficient numbers for the requirements of SIT. By utilising condition-specific promoters to drive expression of the relevant transgenes it is possible to repress the phenotype during rearing until the final generation to be released, simply by switching from repressive to permissive conditions. Conditional expression constructs are a fundamental component in proposed transgenic-based SIT programs such as RIDL (Alphey 2002; Thomas et al. 2000) and transgene-induced embryonic lethality (Horn and Wimmer 2003). Both of these methods have the significant advantage of producing strains that do not require radiation to induce sterility, and thus avoid the associated fitness and production costs.

In the case of RIDL, female-specific dominant lethal genes are used not only as the basis for genetic sexing but also as the agent for population reduction (Alphey 2002). In the wild, released homozygous transgenic males, although not technically sterile, would mate with wild females and their resulting female progeny would die, thereby reducing the reproductive capacity of the population, with the added benefit that their resulting sons, now heterozygous for the transgene would also contribute towards population reduction, in effect doubling the control trait per released male when compared to classical SIT.

Transgene-induced embryonic lethality relies on mass releasing males homozygous for a conditional lethal factor that is active in the early embryo such that, under non-repressive conditions, all matings with transgenic males are effectively sterile since all offspring are inviable (Horn and Wimmer 2003). As proposed, there is no inherent sexing component with such transgene-induced embryonic lethality, but this lethality system could in theory be combined with other existing sexing mechanisms. Transgene-induced conditional embryonic lethality has been shown to work in the Mediterranean fruit fly, where expression of a dominant lethal, driven by a promoter active in the early blastoderm stage of embryo development resulted in effective complete male sterility (Schetelig et al. 2009; Schetelig et al. 2007). While the basic structure of the constructs described in other dipteran insects such as the Mediterranean fruit fly and *Drosophila* is likely to be transferable to *Anopheles*, ultimately the success of any transgenic SIT approach will depend on finding suitable combinations of lethal genes and the regulatory regions necessary to direct their expression.

Candidate genes for causing sterility and female-specific lethality in *Anopheles* mosquitoes

Recently, transgenic *A. gambiae* were generated that effectively expressed complete and dominant embryo-lethality (Windbichler et al. 2008). Expression of a homing endonuclease gene (HEG), which targets repetitive sequences on the

X chromosome, during late stages of spermatogenesis lead to the transfer of stable endonucleases to fertilised embryos, where HEG-induced cleavage of the X chromosome led to early developmental arrest. In theory, the same transgenic construct could be re-engineered so that the endonuclease gene was conditionally expressed, thus rendering mass rearing less problematic. The HEG employed was a fusion protein with GFP, allowing robust sexing of late larvae, in an identical system to the mechano-optical sexing described above (Catteruccia et al. 2005), although this was not performed. If the transgenic construct could be combined with more efficient genetic sexing systems, such strains would fulfil most of the criteria for SIT based on the transgene-induced embryonic lethality approach.

Other promising candidates for lethal genes are pro-apoptotic genes from *D. melanogaster* such as *reaper* (White et al. 1996), *grim* (Chen et al. 1996) and *head involution defective* (Grether et al. 1995), the misexpression of which have all been shown to be lethal in this species. Importantly, the function of these genes seems to be phylogenetically conserved and they induce a similar phenotype across a wide range of species (McCarthy and Dixit 1998; Schetelig et al. 2009; Schetelig et al. 2007), suggesting that they should work in *Anopheles* mosquitoes. With the availability of the *A. gambiae* genome it is possible that other potential pro-apoptotic genes will come to light through homology searching, with potential advantages in potency and efficacy.

With suitable re-engineering it is also possible to make expression of the tTA itself toxic to the insect—by placing the tTA under transcriptional control of its own tetO responsive element, in the absence of tetracycline repression a positive feedback loop is created that is toxic to the cell. The reason for the toxicity is still unknown but could be related to titration of transcription factors away from other essential genes or through upsetting protein homeostasis in the cell. This auto-feedback system of toxicity has been shown to work in both *C. capitata* (Fu et al. 2007; Gong et al. 2005)and *Aedes aegypti* mosquitoes (L.Alphey personal communication) and in principle should be tranferrable to *Anopheles*.

Stability and fitness of transgenic *Anopheles* strains for SIT

The ultimate utility of transgenic strains in full scale area-wide integrated vector management (AW-IVM) programmes will also depend on several factors related to the stability and expression of the transgenic construct and the fitness or viability effects its insertion or expression might confer to the insects both in terms of rearing efficiency and in their mating competitiveness in the wild.

It has been shown that the penetrance of the desired traits, such as embryo lethality (Schetelig et al. 2009) or expression of a sexing marker (Fu et al. 2007), is strongly affected by the localisation of the construct in the genome, through its relation to local enhancers or chromatin patterns that alter transgene expression, phenomena known as position effects. In this context, of promise is the recent successful transfer of the site-specific φC31-mediated AttB/P recombination system in mosquitoes (Nimmo et al. 2006), which allows the site-specific insertion of transgenic constructs to previously characterised chromosomal locations (docking sites). Being able to construct different transgenic containing integrations at identical loci should permit the direct comparison of trait penetrance between different construct designs, without the confounding effects of genome location. This system, already validated in *Aedes aegypti* should, in principle, be transferrable to *A. gambiae*. A scenario in which a site-specific insertion system would be of particular use is the aforementioned construction of genetic sexing strains containing Y-linked transgenes. In general, transposition events on the Y-chromosome are exceptionally rare compared to autosomal insertions, either due to lack of transposition or due to heterochromatic silencing of transposition events. Moreover, given the heterochromatic nature of the Y-chromosome, transgenes inserted there may also be adversely affected by unusually high levels of position effect variegation (Fu et al. 2007) Thus, initially rare Y-linked transgenic docking sites could be chosen, after careful characterisation, for the subsequent generation and comparison of Y-linked transgenic sexing strains.

Potential reductions to the fitness (in terms of mating success) and viability of transgenic SIT mosquitoes could occur as a direct effect of the transgenesis procedure. These effects could result from either transgenic integrations that interefere with endogenous gene function or directly by adverse effects of transgene mis-expression. The fitness of transgenic *Anopheles stephensi* containing a simple fluorescent marker gene was found to be determined as much by genetic bottlenecks introduced in the transgenic breeding scheme as by the presence of the transgene itself (Catteruccia et al. 2003). Obviously, the fitness effects imparted by a transgene will have to be evaluated for each SIT strain constructed. However, in some of the conditional dominant lethal approaches described herein it will be important to minimise 'leakiness' of expression during repressive rearing conditions as this will lead to sub-optimal rearing. In fact, the fitness effect due to the transgene and the stability of the same are inextricable since the selection pressure during mass rearing to de-stabilise the transgene will be proportional to its fitness load.

One concern for transgene instability is the remobilisation of the non-autonomous transposon initially used to insert the transgene by a source of the relevant transposase,

or a closely related transposase, from endogenous transposon copies in the genome. This instability would have to be evaluated for each transposon/genome combination. However, integrated non-autonomous transposons containing transgenes have so far proved very resistant to re-mobilisation in the germline of mosquitoes, even in the presence of the relevant transposase, suggesting a degree of stability (Adelman et al. 2007; Scali et al. 2007; Wilson et al. 2003). Nevertheless, under mass rearing conditions low frequencies of transposon mobilisation that were previously undetectable in the laboratory setting may interfere with strain stability. With this view, methods developed in other insects that delete a terminal repeat sequence of a transposon vector after genomic integration, thus preventing its re-mobilisation (Handler et al. 2004), should be transferable to *Anopheles* species. Ensuring construct immobility will also be crucial in addressing concerns of inter-species movement.

Conclusions

The establishment of a transgenic technology for *Anopheles* mosquitoes and the subsequent refinement of this technology have brought the field to a point where the prospect of transgenic mosquitoes having a role in SIT is a tangible possibility. Future efforts will focus on increasing our knowledge of sex-specific gene expression and in identifying lethal genes and fertility genes with a view to using this information to create new strains for SIT.

There are potential advantages to using a transgenic approach to SIT over the use of 'classical' SIT strains, however each strain will have to be tested on its merits and only rigorous evaluation of a strain's robustness both in the factory and in the field will confirm this. Also, issues related to the public acceptance of new technologies that rely on the release of transgenic material will have to be fully resolved before any transgenic SIT release can become a reality.

Finally, it is unlikely that any SIT strain, however constructed, will be a 'cure all' solution to mosquito control but rather they will form part of a wider integrated pest management program involving several technologies. The relative flexibility of the different methods for achieving sexing and sterility through transgenesis and the speed with which mosquito strains can be constructed should mean that the transgenic approach is ideally suited for producing SIT strains that are likely to be compatible with existing vector control methods.

References

Adelman ZN, Jasinskiene N, Onal S, Juhn J, Ashikyan A, Salampessy M, MacCauley T, James AA (2007) Nanos gene control DNA mediates developmentally regulated transposition in the yellow fever mosquito *Aedes aegypti*. Proc Natl Acad Sci USA 104(24): 9970–9975

Alphey L (2002) Re-engineering the sterile insect technique. Insect Biochem Mol Biol 32(10):1243–1247

Benedict MQ, Robinson AS (2003) The first releases of transgenic mosquitoes: an argument for the sterile insect technique. Trends Parasitol 19(8):349–355

Calkins CO, Parker AG (2005) Sterile insect quality. In: Dyck VA, Hendrichs J, Robinson AS (eds) Sterile insect technique. Principles and practice in area-wide integrated pest management. Springer, Dordrecht, pp 269–296

Catteruccia F, Nolan T, Loukeris TG, Blass C, Savakis C, Kafatos FC, Crisanti A (2000) Stable germline transformation of the malaria mosquito *Anopheles stephensi*. Nature 405(6789):959–962

Catteruccia F, Godfray HC, Crisanti A (2003) Impact of genetic manipulation on the fitness of *Anopheles stephensi* mosquitoes. Science 299(5610):1225–1227

Catteruccia F, Benton JP, Crisanti A (2005) An anopheles transgenic sexing strain for vector control. Nat Biotechnol 23(11): 1414–1417

Chen P, Nordstrom W, Gish B, Abrams JM (1996) Grim, a novel cell death gene in drosophila. Genes Dev 10(14):1773–1782

Curtis CF (1978) Genetic sex separation in anopheles arabiensis and the production of sterile hybrids. Bull World Health Organ (56):453–454

Curtis CF (1979) Genetic sexing techniques based on translocation of insecticide resistance to the y chromosome. Bull OILB/SROP (2)

Curtis CF, Akiyama J, Davidson G (1976) A genetic sexing system in *Anopheles gambiae* species a. Mosquito News (36):492–498

Dame DA, Lofgren CS, Ford HR, Boston MD, Baldwin KF, Jeffery GM (1974) Release of chemosterilized males for the control of *Anopheles albimanus* in el salvador Ii. Methods of rearing, sterilization, and distribution. Am J Trop Med Hyg 23(2): 282–287

Franz G (2002) Recombination between homologous autosomes in medfly (*Ceratitis capitata*) males: type-1 recombination and the implications for the stability of genetic sexing strains. Genetica 116(1):73–84

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. In: Dyck VA, Hendrichs J, Robinson AS (eds) Sterile insect technique. Principles and practice in area-wide integrated pest management. Springer, Dordrecht, pp 427–451

Fu G, Condon KC, Epton MJ, Gong P, Jin L, Condon GC, Morrison NI, Dafa'alla TH, Alphey L (2007) Female-specific insect lethality engineered using alternative splicing. Nat Biotechnol 25(3):353–357

Gong P, Epton MJ, Fu G, Scaife S, Hiscox A, Condon KC, Condon GC, Morrison NI, Kelly DW, Dafa'alla T, Coleman PG, Alphey L (2005) A dominant lethal genetic system for autocidal control of the mediterranean fruitfly. Nat Biotechnol 23(4):453–456

Gossen M, Bujard H (1992) Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. Proc Natl Acad Sci USA 89(12):5547–5551

Grether ME, Abrams JM, Agapite J, White K, Steller H (1995) The head involution defective gene of drosophila melanogaster functions in programmed cell death. Genes Dev 9(14):1694–1708

Grossman GL, Rafferty CS, Clayton JR, Stevens TK, Mukabayire O, Benedict MQ (2001) Germline transformation of the malaria vector, *Anopheles gambiae*, with the piggybac transposable element. Insect Mol Biol 10(6):597–604

Handler AM, Zimowska GJ, Horn C (2004) Post-integration stabilization of a transposon vector by terminal sequence deletion in drosophila melanogaster. Nat Biotechnol 22(9):1150–1154

Holt RA et al (2002) The genome sequence of the malaria mosquito *Anopheles gambiae*. Science 298(5591):129–149

Horn C, Wimmer EA (2003) A transgene-based, embryo-specific lethality system for insect pest management. Nat Biotechnol 21(1):64–70

Kaiser PE, Seawright JA, Dame DA, Joslyn DJ (1978) Development of a genetic sexing system for *Anopheles albimanus*. J Econ Entomol 71(5):766–771

Klassen W, Curtis CF (2005) History of the sterile insect technique. In: Dyck VA, Hendrichs J, Robinson AS (eds) Sterile insect technique. Principles and practice in area-wide integrated pest management. Springer, Dordrecht, pp 3–31

Lycett GJ, Kafatos FC, Loukeris TG (2004) Conditional expression in the malaria mosquito *Anopheles stephensi* with tet-on and tet-off systems. Genetics 167(4):1781–1790

McCarthy JV, Dixit VM (1998) Apoptosis induced by drosophila reaper and grim in a human system. J Biol Chem 273(37): 24009–24015

Nimmo DD, Alphey L, Meredith JM, Eggleston P (2006) High efficiency site-specific genetic engineering of the mosquito genome. Insect Mol Biol 15(2):129–136

Papathanos PA, Windbichler N, Menichelli M, Burt A, Crisanti A (2009) The vasa regulatory region mediates germline expression and maternal transmission of proteins in the malaria mosquito *Anopheles gambiae*: a versatile tool for genetic control strategies. BMC Mol Biol 10:65

Perera OP, Harrell IR, Handler AM (2002) Germ-line transformation of the South American malaria vector, *Anopheles albimanus*, with a piggybac/egfp transposon vector is routine and highly efficient. Insect Mol Biol 11(4):291–297

Rendon P, McInnis D, Lance D, Stewart J (2004) Medfly (diptera: Tephritidae) genetic sexing: large-scale field comparison of males-only and bisexual sterile fly releases in guatemala. J Econ Entomol 97(5):1547–1553

Saccone G, Pane A, Polito LC (2002) Sex determination in flies, fruitflies and butterflies. Genetica 116(1):15–23

Scali C, Nolan T, Sharakhov I, Sharakhova M, Crisanti A, Catteruccia F (2007) Post-integration behavior of a minos transposon in the malaria mosquito *Anopheles stephensi*. Mol Genet Genomics 278(5):575–584

Schetelig MF, Horn C, Handler AM, Wimmer EA (2007) Development of an embryonic lethality system in mediterranean fruit fly *Ceratitis capitata*. In: Vreysen MJB, Robinson AS, Hendrichs J (eds) Area-wide control of insect pest, from research to field implementation. Springer, pp 85–94

Schetelig MF, Caceres C, Zacharopoulou A, Franz G, Wimmer EA (2009) Conditional embryonic lethality to improve the sterile insect technique in *Ceratitis capitata* (diptera: Tephritidae). BMC Biol 7:4

Scott MJ, Heinrich JC, Li X (2004) Progress towards the development of a transgenic strain of the Australian sheep blowfly (*Lucilia cuprina*) suitable for a male-only sterile release program. Insect Biochem Mol Biol 34(2):185–192

Telonis-Scott M, Kopp A, Wayne ML, Nuzhdin SV, McIntyre LM (2009) Sex-specific splicing in drosophila: widespread occurrence, tissue specificity and evolutionary conservation. Genetics 181(2):421–434

Thomas DD, Donnelly CA, Wood RJ, Alphey LS (2000) Insect population control using a dominant, repressible, lethal genetic system. Science 287(5462):2474–2476

White K, Tahaoglu E, Steller H (1996) Cell killing by the drosophila gene reaper. Science 271(5250):805–807

Wilson R, Orsetti J, Klocko AD, Aluvihare C, Peckham E, Atkinson PW, Lehane MJ, O'Brochta DA (2003) Post-integration behavior of a mos1 mariner gene vector in *Aedes aegypti*. Insect Biochem Mol Biol 33(9):853–863

Windbichler N, Papathanos PA, Crisanti A (2008) Targeting the x chromosome during spermatogenesis induces y chromosome transmission ratio distortion and early dominant embryo lethality in *Anopheles gambiae*. PLoS Genet 4(12):e1000291