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**REVIEW**

# Genetic Improvements to the Sterile Insect Technique for Agricultural Pests

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**Abstract.** The sterile insect technique (SIT) relies on area-wide mass-releases of sterile male pest insects, which mate with their wild counterparts and thereby cause a drop in the wild population. In order to improve SIT efficacy or to avoid potential negative effects of such releases, strains of insects have been developed by genetic means. Methods of strain improvement fall into two categories: those generated by classical genetics and those through transgenesis. Here, we describe development and successes of agriculturally important pest insect strains developed through the former, and how transgenic technology is offering a broad spectrum of potential improvements to SIT in a wider range of insects. Also discussed are future prospects and non-technical challenges faced by transgenic technology. The need for environment-friendly pest control methods in agriculture has never been more pressing. SIT and related technologies offer a solution with proven effectiveness.

**Keywords:** Agriculture; Sterile Insect Technique (SIT); Transgenic; Pest; Genetic Sexing.

## INTRODUCTION

The Sterile Insect Technique (SIT), in which very large numbers of sterilised insects are released to reduce mating between their fertile wild counterparts, was first proposed by Knipling in 1955 (Knipling, 1955). Even earlier, Serebrovskii put forward the idea of releasing genetically mutated insects for a similar purpose: to spread deleterious mutations into the wild population and thereby reduce it (Serebrovskii, 1940). It has since been used to control and eradicate populations of agricultural and veterinary insect pests around the world. As with other area-wide pest management methods, SIT is most effective when applied over a broad spacial area, rather than treating fields or locations individually (Klassen, 2005).

Notable successes, in which sterile males and females were mass-released – and in many cases still are – include the eradication of the New World screwworm (NWS, *Cochliomyia hominivorax* Coquerel) from North and Central America (reviewed in Klassen and Curtis, 2005), and from Libya (FAO, 1992; Lindquist *et al.*, 1992); the tsetse fly (*Glossina austeni* Newst.) from Unguja Island in Zanzibar, Tanzania (Vreysen *et al.*, 2000); the melon fly (*Bactrocera cucurbitae* Coquillett) from Japan (Kuba *et al.*, 1996) and the Queensland fruit fly (*Bactrocera tryoni* Froggatt) from western Australia (Sproule *et al.*, 1992). SIT has prevented establishment of the Mediterranean fruit fly (medfly, *Ceratitis capitata* Wied.) in California and Florida, USA (Dowell *et al.*, 2000; Barry *et al.*, 2004), Mexico (Hendrichs *et al.*, 1983) and Chile (Esparza Duque, 1999; Gonzalez and Troncoso, 2007). Similarly, regular sterile releases of pink bollworm (*Pectinophora gossypiella* Saunders) have inhibited the long-term establishment of this pest in the San Joaquin Valley, California (Staten *et al.*, 1999). In 2005, a programme was initiated that set out to eradicate the pink bollworm from

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south-western USA and adjacent areas of northern Mexico (El-Lissy *et al*., 2003; Smith *et al*., 2004), with sterile releases of moths as a key component of the strategy (Henneberry and Naranjo, 1998).

Even apart from these instances of eradication based on SIT, in many regions SIT is successfully applied to suppress the population of established pest species: for example, medfly in Guatemala, codling moth (*Cydia pomonella* L.) in Canada and the West Indian sweet potato weevil (*Euscepes postfasciatus* Fairmaire) in Japan, to name just a few.

Accepting that SIT can be successful for pest control, this method provides further benefits. It is species-specific; unlike chemical control it has no off-target effects on the environment or on human health. Consequently, it is also compatible with organic agricultural practices (Wimmer, 2005; Hendrichs *et al*., 2007). In addition, because released insects are mobile and would actively seek mates, control of a pest can be effective on an area-wide basis, beyond the range of its immediate application.

In its long history, SIT has been associated with genetic advances to improve its effectiveness. These are described here, from the development of early genetic sexing strains that allowed for release of males only, to the spectrum of benefits that transgenic technology might bring.

## IMPROVEMENT OF SIT FOR AGRICULTURE BY CLASSICAL GENETICS

Although highly successful area-wide SIT programmes have been conducted by the simultaneous release of irradiated males and females (Lindquist *et al*., 1992; Wyss, 2000), for many agricultural pests it would be highly preferable to eliminate females from the release population. This is for several reasons. Firstly, sterile females are commonly found ovipositing and thus cause serious economic damage by so-called sting damage to fruit and consequent fungal and viral infections. Secondly, sterile females divert sterile males from finding and mating fertile wild females. Even if females are neutral to SIT effectiveness in the field, they can significantly add to rearing, handling and distribution costs (Cáceres, 2002), therefore undermining the general efficacy of an operational SIT programme. Of course, for SIT against disease vectors it is frequently the blood-feeding females that transmit infections, which provides an additional reason to prefer male-only releases.

***Genetic sexing strains in the Australian sheep blowfly*** In 1969, Whitten put forward the idea of generating genetic sexing strains (GSS) using male-linked chromosomal translocations (Whitten, 1969). This method was first, and briefly, employed in the Australian sheep blowfly (*Lucilia cuprina* Wied.) in a small SIT trial in 1972-1973 (Whitten & Foster, unpublished data; reported in (Robinson, 2002a)), in which a pupal colour mutation was used to separate sexes before emergence and allow male-only releases. With another GSS, suppression of a *L. cuprina* population was shown in two field trials carried out in 1984-1986 (RJ Mahon, TL Woodburn & GG Foster, unpublished; reported in Foster *et al*., 1991; Mahon, 2001). In this female-killing (FK) system, females were homozygous for eye-pigment mutations and had white eyes. This lack of eye pigment rendered them blind, thereby resulting in death in the field (Whitten *et al*., 1977). The males of FK strains also carried these mutations on autosomes, but additionally had a wild-type allele on a Y-chromosome translocation. With their normal eye pigmentation, they were competitive in the field and able to find mates. Such pairings between FK males and wild females resulted in a drop in the fertility of the blowfly population due to the translocation imparting semi-sterility and increasing frequency of homozygous mutants (resulting in female blindness and death) after sustained releases of the GSS (Whitten, 1969; Whitten *et al*., 1977; Foster *et al*., 1985). One advantage of this approach is that irradiation, which may add fitness and financial costs (discussed in Robinson *et al*., 2004), is not required. On the other hand, the mutations and chromosome rearrangements used resulted in a high level of sterility and consequent difficulties in rearing the strain in large numbers for field release. The FK strains were also unstable as a result of their complex genetic rearrangements. During mass-rearing the strain lost the mutations and reversion of the translocation back to a free Y chromosome was observed, with the result that, over time, fewer eye colour mutations were introduced into the wild population and increasing numbers of fully fertile males were released. Unfortunately, these factors, as well as a reduction in funding due to falling wool prices, contributed to a decision not to progress to full-scale SIT with *L. cuprina*.

***Genetic sexing strains in medfly: a global success*** Following this pioneering work in *L. cuprina*, most GSS development has been conducted in the medfly, based on the same two-component strategy: a selectable recessive marker that can be used for sorting or killing of the females; and a Y-autosome translocation linking the dominant wild-type allele of this marker to the male sex. Based on these two components, strains can be generated where males and females are sufficiently different from each other so that they can be automatically separated on a large scale: the females are homozygous for the selectable marker and therefore mutant, while the males show the wild phenotype.

The first medfly GSS were constructed in 1984 by combining Y-autosome translocations developed by Robinson and van Heemert (1982) with the white pupae mutation (*wp*, located on chromosome 5) previously detected by (Rössler, 1979) (Figure 1). One particular strain, T(Y;5)101, was selected to develop the first small-scale mass-rearing. Initial laboratory tests showed that the strain is less fertile than the wild type control, which is to be expected because of the translocation present in the males, but otherwise the strain showed no abnormalities and, in particular, no genetic instability was observed. However, when the strain was mass-reared to produce ~1 million males/week, it showed

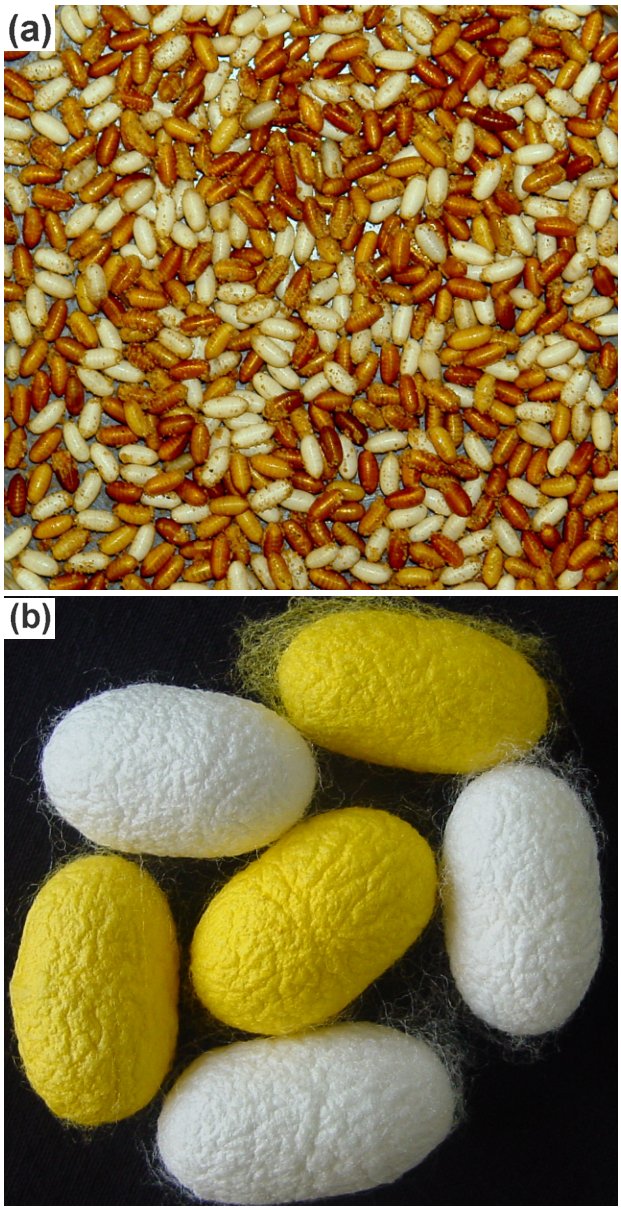


**Figure 1. Mutations as visible markers.** Mutations used as visible markers in **(a)** medfly, the male pupae showing a wild-type brown pupal phenotype and the females exhibiting the mutant white pupal phenotype; and **(b)** silkmoth, with the yellow cocoon colour gene translocated from chromosome 2 to the W chromosome (Tazima *et al.*, 1951): male cocoons of such a strain are white and female cocoons are yellow.

significant levels of genetic instability, which increased over time, revealed by a marked increase in $wp^+$ females (i.e. females emerging from brown pupae) (Busch-Petersen and Kafu, 1989). These females could not be distinguished from the males and, consequently, an increasing number of females were to be released into the field. The cause for this is male recombination in the chromosomal region between the translocation breakpoint and the location of the selectable marker(s) (Type-1 recombination) (Franz, 2002). In each generation around 0.1% recombinant *wp* males and $wp^+$ females are produced, depending on the strain used. In the highly selective environment of mass-rearing the $wp^+$ females accumulate in the colony, not in an additive fashion, but faster because they have a selective advantage over their non-recombinant sisters.

These results illustrate how difficult it can be to predict the outcome of large-scale rearing results based on very small populations raised in the laboratory. Some of the genetic phenomena responsible for the breakdown of GSS are so rare that they cannot be detected in small-scale rearing and, secondly, small-scale rearing is not selective enough to cause the rapid accumulation of the recombinants. In addition to these results, together with the stability problems of the *L. cuprina* strains, gave the entire technology a bad reputation, and the scepticism about its use in operational programmes persisted, in some cases for nearly 20 years.

The underlying concept of male-only releases was disputed for a long time. Based on the principle of the SIT it is agreed that the primary target of the SIT is the wild female because the number of fertile eggs it produces determines the size of the population. The number of fertile eggs can be reduced by the mating of the wild females with the released sterile males. However, some argued that the released sterile females also play a role in the SIT by serving as a "sponge" for the wild sperm and thereby reducing the fertilization of the wild females (Whitten and Taylor, 1970; Robinson *et al.*, 1999). Proponents of the male-only concept, on the other hand, point to the fact that the wild males (more precisely their sperm) are present in such a vast excess that the removal of a very large proportion of the wild males would be required before any impact on the population size could be achieved. However, this is unrealistic considering the sex ratio of the released flies. This view is supported by two independent lines of evidence. In the Male Annihilation approach, used against some other tephritid fruit flies where very potent male attractants are available, 99% of the wild males have to be killed before a reduction of population size becomes apparent (Koyama *et al.*, 1984). Secondly, several field tests of various scales showed that male-only releases are superior (reviewed by Robinson *et al.*, 1999). In particular the tests by McInnis and colleagues (1994) provided important insight. They compared bi-sexual, female-only and male-only releases and clearly showed that females alone do not generate any reduction of the wild population, while male-only releases introduced 3-4 times more sterility into the target population than bi-sexual releases. Later, Rendón and colleagues found a similar effect (3-5 times more sterility per released male) in very large scale release experiments (Rendón *et al.*, 2004). Additional benefits of GSS in operational programmes are reviewed by Hendrichs *et al.* (1995).

From the results obtained with the first generation of medfly GSS the following conclusions were drawn: first, releasing only males into the target population is clearly more efficient than bi-sexual releases. However, the sexing principle, based on selectable markers allowing sorting of male and female pupae, is not really suitable for large-scale mass-

rearing because (a) the sexing takes place after the expensive mass-rearing and also creates the problem of disposal of large quantities of female pupae; (b) it requires expensive sorters that require frequent calibration and servicing; (c) the sorting shows only 95-97% accuracy; (d) male production efficiency is reduced because a considerable proportion of the males is removed together with the females; and (e) male quality is reduced as the sorting procedure leads to a reduction in flight ability. Furthermore, the first-generation GSS were not stable enough to be acceptable for operational programmes.

Based on these conclusions a new selectable marker, a temperature-sensitive lethal mutation (*tsl*), was isolated. With this marker the homozygous females can be eliminated at the earliest possible stage, i.e. during embryogenesis, by incubating eggs in a 34°C water bath (Franz *et al*., 1996). Individuals heterozygous for a *tsl* wild type allele, like the males in a GSS, are not affected by this treatment provided that the treatment is done during later stages of embryogenesis to avoid maternal effects (Franz, 2005). *tsl* is closely linked to *wp* (Figure 2a), which is convenient for the induction of new translocations and also later on for the quality control of the GSS (the two traits are only infrequently separated by translocations). Secondly a whole series of new translocations was induced. These were analysed to identify those where (a) only one autosome is involved to avoid high sterility levels associate with complex translocations; and (b) the distance between the translocation breakpoint and the chromosomal location of the *tsl* is small to minimise Type-1 recombination (Franz *et al*., 1994). Over the following years several additional measures were taken to ensure the stability of the GSS. It was, for example, discovered that the breakpoint on the Y chromosome is also important for the stability of the GSS, as well as for the sterility associated with the segregation behaviour of translocation during male meiosis. A breakpoint close to the Y-chromosomal centromere reduces recombination between the two translocated Y fragments (Type-2 recombination; Franz, unpublished). Furthermore, a pericentric inversion on chromosome 5 was included in the strains to further reduce Type-1 recombination. In addition, the rearing process was revised by replacing the traditional continuous mass-rearing with a Filter Rearing System (FRS) (Fisher and Caceres, 2000). In the past a certain fraction of the production (ca. 20%) was used to set up the cages for the next generation. Carried out continuously for many years, this practice allowed recombinants to accumulate in the colony due to their selective advantage. In the FRS a clean stream is maintained free of recombinants by screening each generation. From this clean stream three consecutive amplification steps (generations) are required to increase the colony as required for desired male-only production level. The FRS is a one-way strategy, i.e. the flow of material is always from the clean stream towards to production colony. Consequently, the accumulation of recombinants is limited because it is restricted to three generations of mass-rearing. Both strategies together - improvement of the stability of the strains and the FRS - make it possible that the stability and accuracy of sexing can be maintained at a very high level (e.g. 99.75%) over many years, i.e. instability of the GSS is no longer a problem even in facilities producing more than 1000 million males per week (Dyck *et al*., 2005).

In 1994 the first second-generation GSS was introduced into the mass rearing facility in Petapa, Guatemala (Franz *et al*., 1996). After several years of evaluations and after adapting the rearing process to the special requirements of the strain it was transferred to the newly built facility in El Pino, Guatemala. In subsequent years all medfly mass-rearing facilities replaced their bi-sexual strains with GSS and all facilities built in the intervening years, for example in Portugal, Israel, Spain and Tunisia, adopted GSS immediately. The combined maximum production capacity of all medfly mass-rearing facilities world-wide is ~3500 males per week (G. Franz, personal communication).

An alternative sexing strain has been developed in which females can be separated due to a slower rate of development time (Cladera and Delprat, 1995). This is already possible to some extent, though, in current *tsl*-based GSS: in mass-rearing the first day of pupal collection yields mostly males; the fifth day yields mostly females (Franz, 2005).

GSS technology has also been extended to other fruit-fly species – the melon fly (*Bactrocera cucurbitae* Coquillett) (McInnis *et al*., 2004), *Bactrocera dorsalis* (Hendel) (McCombs and Saul, 1995) and the Queensland fruit fly (*Bactrocera tryoni* Froggatt) (Meats *et al*., 2002) – but these have not yet been used on a commercial scale.

***Genetic sexing strains in Lepidoptera*** Similar GSS have been developed in the silk worm, *Bombyx mori*, in which sex-linked visible markers can be used to separate the sexes. This, clearly, is not for the purpose of pest control as silk worm is not a pest, rather males and females are required to be separated to make F1 hybrids since only hybrids are reared in large scale silk production (Nagaraju *et al*., 1996). Availabilty of GSS in sericulture obviates the need for manual sex separation of millions of cocoons used in hybrid preparation thus saving labour cost and wastage of cocoons. Besides, males are favoured in sericulture as they produce more silk and consume less diet than females (Nagaraju, 2002). The yellow cocoon colour gene on chromosome 2 (Figure 1b) and the black egg colour gene on chromosome 10 have both been translocated to the female-determining W chromosome (Tazima *et al*., 1951; Kimura *et al*., 1971). Perhaps more useful strains are those that follow the scheme initially proposed by Strunnikov in 1975 (Strunnikov, 1975; reviewed in Marec *et al*., 2005), so-called balanced-lethal (BL) strains. BL males are trans-heterozygous for two sex-linked recessive lethal mutations (SLRLMs) on the Z chromosomes, and BL females carry one of the two SLRLMs as well as a portion of the Z chromosome translocated to the W chromosome (Figure 2b). As this translocation also carries the wild-type alleles of the two SLRLMs, the female survives. Half of male progeny perish in rearing due to their being homozygous for one of the SLRLMs; the other surviving half are protected due to their heterozygous, 'balanced',

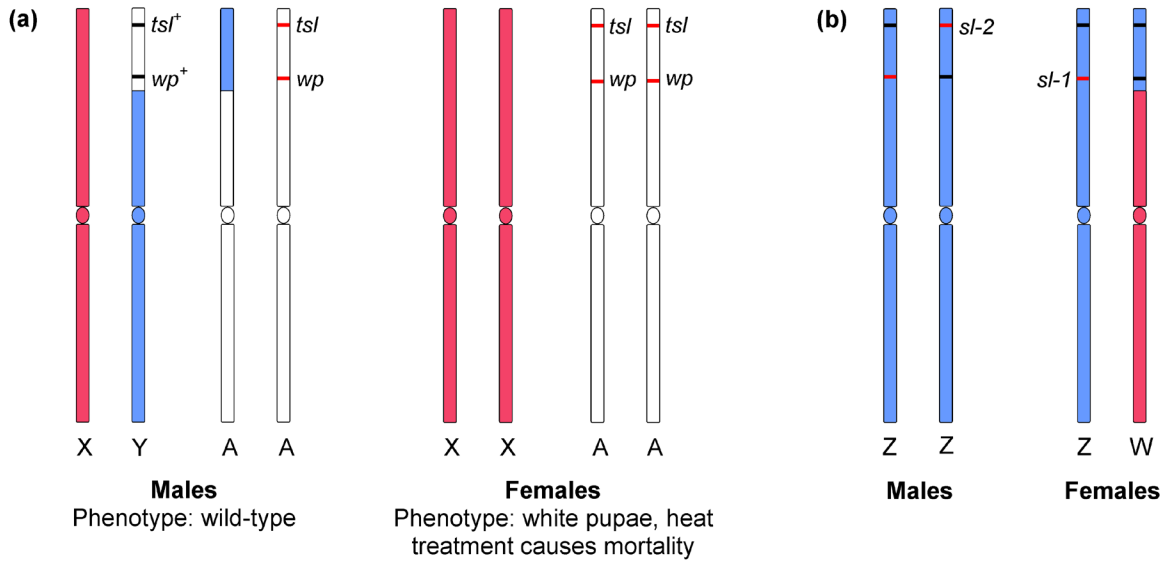


**Figure 2. Schematic diagram showing the basic structures of GSS chromosomal translocations in (a) medfly and (b) the Mediterranean flour moth.** **(a)** In medfly GSS males (Franz, 2005), the wild-type alleles of *temperature sensitive lethal* (*tsl*) and *white pupae* (*wp*) (marked as black) are translocated to the Y chromosome (shown predominantly blue), with a reciprocal translocation to the autosome. The remaining, intact autosome carries the mutant alleles of *tsl* and *wp* (marked as red). In females, which lack the Y chromosome, two copies each of mutant *tsl* and *wp* are present, conferring the mutant phenotypes that allow for male selection. **(b)** In moth balanced-lethal strains (Marec *et al.*, 2005), males carry one copy each of the recessive lethal mutations, *sl-1* and *sl-2*, but on different Z chromosomes. Females, which are the hemi-zygous gender in most Lepidoptera, carry only the *sl-1* mutation on a W chromosome translocation to their Z chromosome, with their W chromosome carrying the wild-type alleles for the mutations. To maintain a stock of such a strain, the males and females of the genotypes shown here are crossed together. Only the males that inherit two copies of the mutant *sl-1* die (around half of males). To initiate male selection, the males shown below are crossed with wild-type females. The female progeny inherit the *sl-1*-carrying Z chromosome from the father, lack the translocation on the W chromosome carrying wild-type *sl-1* allele, and therefore die; whereas the male progeny inherit a wild-type Z chromosome (carrying the non-mutant *sl-1* allele) from the mother, as well as the mutant *sl-1* allele from the father, and survive.

state. Sexing (female death) is achieved by crossing BL males to wild-type females: all male progeny survive due to rescue by the wild-type alleles of the SLRLMs; all females die as the wild-type W chromosome they inherit does not carry the SLRLM-rescuing portion of the Z chromosome.

This GSS method was extended to a pest lepidopteran, the Mediterranean flour moth (*Ephestia kuehniella* Zeller) (Marec, 1991; Marec *et al.*, 1999). In the BL-2 strain, males are balanced for two SLRLMs, sl-2 and sl-15, and females carry the wild-type alleles of these loci on their W chromosome. Sexing is initiated by crossing BL-2 males as described for *B. mori* BL strains described above, resulting in 99.7% males in the progeny (females represented recombinants) (Marec *et al.*, 1999). These promising results are offset by the fact that two strains (BL and wild-type) would be required in mass-rearing, and out-crossing BL males to wild-type females in such large numbers might be considered prohibitive for SIT application. Strain instability, through genetic recombination, is also a clear disadvantage to rearing this strain: as with fruit flies, close monitoring in a FRS would be required.

## POSSIBLITIES FOR SIT IMPROVEMENT BY TRANSGENESIS

Levin (1979) proposed genetic engineering as a solution to pest-related problems at the Rockefeller Foundation (Robinson, 2002b). At that time the possibility of introducing new genes to the genome of insect pests seemed remote. Nevertheless, in 1982, Rubin and Spradling successfully transformed *Drosophila melanogaster* using *P* element transposition (Rubin and Spradling, 1982), opening the way to the use of this new technology to pest control. Early attempts to transform pest insects using *P* element vectors failed, however (but see Miller *et al.*, 1987), it was soon recognised that the *P* element system was restricted to drosophilids (Handler *et al.*, 1993; O'Brochta and Atkinson, 1996; Atkinson *et al.*, 2001). Another major problem associated with these failures

was the lack of efficient and versatile transformation markers (Hagler and Jackson, 2001). Use of the *Minos* element allowed reproducible stable germ line transformation of *C. capitata*, using a *white*$^+$ cDNA eye colour marking system (Loukeris *et al*., 1995; Zwiebel *et al*., 1995). Identification of new transformation systems allowed genetic engineering of diverse pest insects and allowed for the development of new strategies to improve SIT. *Mariner, Minos, Hermes* and *piggyBac* have been the most widely used transposable elements to date and have allowed transformation of several species of Diptera, Hymenoptera, Lepidoptera and Coleoptera (Table 1). Transgenic technology may enhance at least three aspects of operational SIT programmes: monitoring, genetic sexing and sterilisation. Discussed here are such developments in tephritids and Lepidoptera, in which by far the majority of such transgenic work in agricultural pests has been undertaken.

***Genetic marking by transgenesis*** Germline transformation requires a selectable marker. Fluorescent proteins have been used for this purpose in the vast majority of transgenesis work on pest insects. Expression of these proteins, under the control of suitable regulatory sequence, provides a readily distinguishable marker for the transgenic insect. From an SIT perspective, another key feature is that such markers are in-built and heritable.

Current marking techniques (Hagler and Jackson, 2001; Parker, 2005) mainly involve the use of coloured dyes in the larval diet that remains visible in the adults' tissues (e.g. codling moth, pink bollworm), or application of powder directly to the pupae (medfly). Although widely used, for example the Calco Red dietary dye for pink bollworm (*Pectinophora gossypiella*) moths (Graham and Mangum, 1971) and fluorescent powders marking the heads of adult medfly (Steiner, 1967), their application increases the cost of rearing, can increase the amount of handling required, may pose a risk to worker health, and is prone to errors of interpretation (Hagler and Jackson, 2001; Hagler and Miller, 2002; Robinson and Hendrichs, 2005). It is also possible that a fraction of released sterile insects lose the marker after release (Hagler and Jackson, 2001; Hagler and Miller, 2002), which would mean that on recapture they may be counted among the wild and fertile insects in the traps. Such error would not have a significant impact where large numbers of wild insects are captured, but in programmes attempting to eradicate a pest and where the wild pest is relatively infrequently captured, the presence of one such insect might provoke a costly round of quarantine and exceptional interventions.

For some species, full sterilisation by irradiation is achieved at a dose that negatively affects the performance of insects (Bakri *et al*., 2005). In SIT programmes against these species the applied dose is therefore not fully sterilising in order to minimise this effect, even though this means releasing some fertile or partially fertile insects. The fact that dye or powder markers are not heritable leads to the possibility that recaptured progeny of such 'sterile' insects with wild counterparts will be scored as wild.

A dominant, heritable fluorescent marker conferred through transgenesis (Berghammer *et al*., 1999; Peloquin *et al*., 2000; Pinkerton *et al*., 2000; Tamura *et al*., 2000; Handler and Harrell, 2001a; Horn *et al*., 2002; Perera *et al*., 2002; Allen *et al*., 2004; Catteruccia *et al*., 2005; Koukidou *et al*., 2006) would help to avoid the above problems: no extra cost or handling would be required for marking. Strongly fluorescent strains (e.g. Figure 3) might be expected to avoid the occasional false-negative insect on traps. As with *tsl*-based GSS in medfly (San Andrés *et al*., 2007), PCR-based identification of the transgene insertion could act as a highly reliable secondary method of marker detection.

A complementary tool for male-only releases of insects is the ability to distinguish between sperm from released insects and that from fertile wild insects. This allows the source of sperm in captured wild females to be determined, which allows the programme managers to monitor how well released males are transferring sterile sperm irrespective of their mating competitiveness. It is not impossible to do this without transgenic methods as one can look for morphological differences between irradiated and unirradiated sperm in the captured females' spermathecae, examine the progeny of live-trapped females, or measure the survival of eggs recovered from the wild, however these methods are laborious and cumbersome. Using a promoter sequence from medfly beta-tubulin (a gene involved in spermatogenesis), Scolari *et al*. (2008) were able to engineer sperm-specific expression of the DsRed and EGFP fluorescent proteins in the testes of medfly strains (Scolari *et al*., 2008). Similar strains have been produced for both *Anopheles stephensi* (Catteruccia *et al*., 2005) and *Aedes aegypti* mosquitoes (Smith *et al*., 2007). The fluorescence in testes may allow separation of the sexes by screening, and for transgenic males to be distinguished from wild-type flies in field traps. The problems associated with automated sex-sorting by colour/fluorescence, as described for medfly pupal colour mutants earlier in this article, mean that this would not be a practical method of selecting males in large-scale mass-rearing, though it might have some value in a filter colony. Maybe more usefully, dissection of wild-type females that have mated with such males reveals spermathecae with fluorescent sperm.

In Lepidoptera, the progeny of insects given a sub-sterilising dose of irradiation are themselves sterile; an effect known as F1 sterility (Vreysen *et al*., 2010). Use of this characteristic has potential as a tool for SIT improvement: with a reduced radiation dose, the released insects' performance would be improved. Any progeny of these released insects would be fully sterile and, not having been irradiated, might be expected to compete well for mates. Being heritable, a transgene marker would allow such F1 progeny to be detectable and distinguishable from wild insects.

***Transgenesis as a means to achieve radiation replacement*** Sterilising insects by irradiation can have a negative impact on their performance in the field. The magnitude of this problem is controversial and difficult to define (Robinson *et al*., 2004). However, any reduction in insect qual-

**Table 1.** Summary of transposable element-mediated stable germline transformation of non-drosophilid insect species.

| Family | Species name(s) | Transposable element | Reference |
|---|---|---|---|
| **Mosquitoes** | | | |
| Culicidae | Yellow fever mosquito, *Aedes aegypti* | *Mariner* | (Coates *et al.*, 1998) |
| | | *Hermes* | (Jasinskiene *et al.*, 1998) |
| | | *piggyBac* | (Kokoza *et al.*, 2001) |
| | Asian tiger mosquito, *Aedes albopictus* | *piggyBac* | (Labbé *et al.*, 2010) |
| | *Aedes fluviatilis* | *piggyBac* | (Rodrigues *et al.*, 2006) |
| | New World malaria mosquito, *Anopheles albimanus* | *piggyBac* | (Perera *et al.*, 2002) |
| | African malaria mosquito, *Anopheles gambiae* | *piggyBac* | (Grossman *et al.*, 2001) |
| | Indo-Pakistan malaria mosquito, *Anopheles stephensi* | *Minos* | (Catteruccia *et al.*, 2000) |
| | | *piggyBac* | (Ito *et al.*, 2002; Nolan *et al.*, 2002) |
| | Southern house mosquito, *Culex quinquefasciatus* | *Hermes* | (Allen *et al.*, 2001) |
| **Fruit flies** | | | |
| Tephritidae | Mexican fruit fly, *Anastrepha ludens* | *piggyBac* | (Condon *et al.*, 2007b) |
| | Caribbean fruit fly, *Anastrepha suspensa* | *piggyBac* | (Handler and Harrell, 2001b) |
| | Oriental fruit fly, *Bactrocera dorsalis* | *piggyBac* | (Handler and McCombs, 2000) |
| | Olive fly, *Bactrocera oleae* | *Minos* | (Koukidou *et al.*, 2006) |
| | Queensland fruit fly, *Bactrocera tryoni* | *piggyBac* | (Raphael *et al.*, 2010) |
| | Mediterranean fruit fly, *Ceratitis capitata* | *piggyBac* | (Handler *et al.*, 1998) |
| | | *Hermes* | (Michel *et al.*, 2001) |
| | | *Minos* | (Loukeris *et al.*, 1995) |
| **Other Diptera (pest, myiasis, biting flies)** | | | |
| Muscidae | Housefly, *Musca domestica* | *piggyBac* | (Hediger *et al.*, 2001) |
| | | *Mariner* | (Yoshiyama *et al.*, 2000) |
| | Stable fly, *Stomoxys calcitrans* | *Hermes* | (O'Brochta *et al.*, 2000) |
| Calliphoridae | Australian sheep blowfly, *Lucilia cuprina* | *piggyBac* | (Heinrich *et al.*, 2002) |
| | New World screwworm, *Cochliomyia hominivorax* | *piggyBac* | (Allen *et al.*, 2004) |
| **Wasps, bees and ants** | | | |
| Hymenoptera | Sawfly, *Athalia rosae* | *piggyBac* | (Sumitani et al., 2003) |
| **Beetles** | | | |
| Coccinellidae | Harlequin ladybird, *Harmonia axyridis* | *piggyBac* | (Kuwayama *et al.*, 2006) |
| Tenebrionidae | Red flour beetle, *Tribolium castaneum* | *piggyBac & Hermes* | (Berghammer *et al.*, 1999) |
| | | *Minos* | (Pavlopoulos *et al.*, 2004) |
| **Butterflies and moths** | | | |
| Nymphalidae | Squinting bush brown butterfly, *Bicyclus anynana* | *piggyBac & Hermes* | (Marcus *et al.*, 2004) |
| Gelechiidae | Pink bollworm, *Pectinophora gossypiella* | *piggyBac* | (Peloquin *et al.*, 2000) |
| Bombycidae | Silkworm, *Bombyx mori* | *piggyBac* | (Tamura *et al.*, 2000) |
| | | *Minos* | (Uchino *et al.*, 2007) |

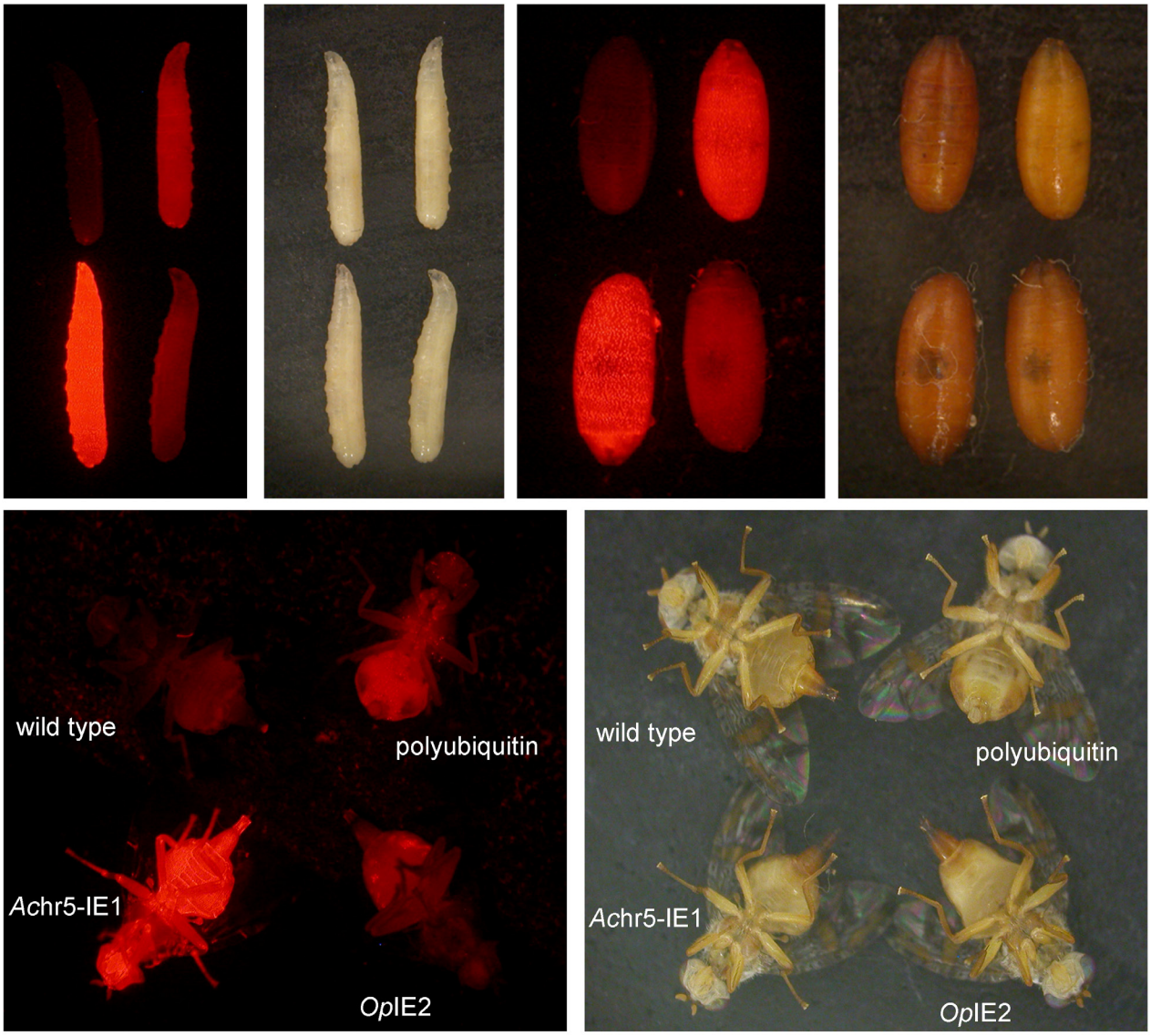


**Figure 3. Fluorescent protein markers in transgenic strains of medfly.** Larvae, pupae and adults from a wild-type strain or transgenic strains marked with DsRed driven by a polyubiquitin promoter, a baculovirus promoter, *Achr5*-IE1, or another baculovirus promoter, *OpIE2* (strains and photographs courtesy of P. Gong, G.C. Condon, K.C. Stainton and N.I. Morrison).

ity results in a requirement for releases of larger numbers of insects to compensate for this performance deficit, and therefore higher operational costs. Transgenesis has great potential as a means of addressing this issue: by using genetic systems to induce death at given life stages, the effect of sterility might be achieved without the need for irradiation and related steps that require further handling of the insects. Genetic systems that cause lethality or sterility must be tightly regulated so that mass-reared colonies can be reared efficiently and performance in the field is not compromised. The best-known of these are the tetracycline-regulated systems (Gossen and Bujard, 1992; Gossen *et al*., 1994; Gossen and Bujard, 2002). In the absence of tetracycline, the synthetic transactivator (tTA) activates gene expression through binding to a multimer of a binding site (*tetO*) placed up stream of a minimal promoter. In the presence of tetracycline, tTA undergoes conformational change that prevents it from binding to the *tetO* sites, thereby preventing expression of the target gene (Gossen and Bujard, 2002). A major advantage of the Tet system is that two alternative tTAs exist that are affected by tetracycline or analogues in the opposite manner; the original transactivator is inactivated by tetracycline, thus preventing expression (called here the 'Tet-off' system), whereas the reverse transactivator (Urlinger *et al*., 2000) is activated by tetracycline ('Tet-on' system). Both systems have been used across a very wide phylogenetic range and were first used in *Drosophila* by Bello *et al*. (1998).

Thomas *et al*. (2000) proposed the Release of Insects carrying a Dominant Lethal (RIDL®), in which the dominant lethal gene – introduced by transgenesis – induces death of

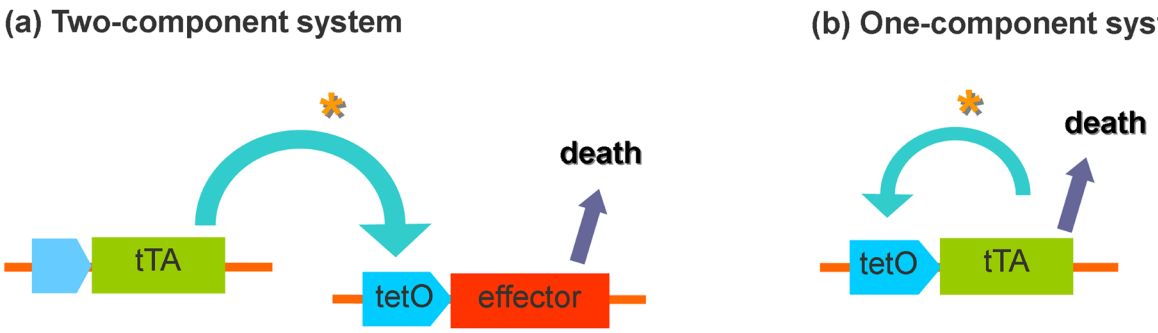


**Figure 4. Tetracycline-repressible lethal systems. (a)** Two-component system as previously published (Heinrich and Scott, 2000; Thomas et al., 2000; Horn and Wimmer, 2003). tTA is placed under the control of a suitable promoter, e.g. constitutive, female-specific, embryo-specific, etc. In the absence of tetracycline (Tc), tTA binds *tetO*, drives expression of an effector molecule leading, in the case of a lethal effector, to death. In the presence of Tc, tTA binds Tc*; the Tc-bound form does not bind DNA, therefore does not activate expression of the effector, and the system is inactivated. **(b)** A simplified one-component system (Gong *et al.*, 2005). In the absence of Tc, basal expression of tTA leads to the synthesis of more tTA, which accumulates to high level. This level can be regulated by modifying the stability and translational efficiency of the tTA mRNA. At the highest levels, expression is lethal, so tTA is both the driver and the effector. In the presence of Tc, tTA is inactivated by Tc* and is therefore expressed only at basal levels.

the insects (in this case, females only) before adulthood, and preferably much earlier. The lethal gene could be repressed by the Tet-off system in permissive conditions: in the presence of a dietary additive (tetracycline or suitable analogues) larvae survive to adulthood. Such RIDL strains could therefore be mass-reared happily, with female lethality being introduced by withholding tetracycline from the larval diet of the pre-release generation (Alphey, 2002; Alphey, 2007; Alphey *et al.*, 2008). The permissive conditions will not, of course, be encountered in the wild, so such strains would confer lethality in female progeny after mating with wild counterparts, and are self-limiting after release.

The first reported RIDL pest strains were the medfly lines generated by Gong *et al.* (2005), this time inducing bi-sex lethality. Males and females of these medfly strains were viable and fertile when reared on tetracycline. In the absence of the dietary additive, however, both males and females died as larvae or pupae; time of death depends on the construct and strain. In this one-gene tetracycline-repressible system (Figure 4), tTA is used as both a transactivator and an effector: in the absence of tetracycline, tTA levels become deleterious to cells, probably due to transcriptional 'squelching' or interference with ubiquitin-dependent proteolysis (Gill and Ptashne, 1988; Berger *et al.*, 1990; Gossen and Bujard, 1992; Damke *et al.*, 1995; Salghetti *et al.*, 2001). Although embryos are able to develop to larvae, for the purposes of controlling the pest population, the effect of this larval mortality is the same as strict, agametic sterility: no offspring survive to reproduce. Work towards generating similar strains of pink bollworm has also been reported (Simmons *et al.*, 2007), and equivalent repressible bi-sex lethality has been achieved in *Aedes aegypti* (Phuc *et al.*, 2007; reviewed in Wilke *et al.*, 2009; Alphey *et al.*, 2010).

Horn and Wimmer (2003) demonstrated in *D. melanogaster* another version of this approach to inducing reproductive sterility. Using a two-part tetracycline-repressible system (Figure 4), sterility was induced by a transgene combination that caused dominant embryo-specific lethality in the progeny. The authors used enhancer-promoter sequences of the *serendipity a* (*srya*) and *nullo* blastoderm genes in association with the pro-apoptotic gene *head involution defective* (hid) as an effector component. Although this embryonic lethality was successfully achieved in *Drosophila*, a direct transfer of this system to medfly was not successful. However, in the same laboratory, the use of newly isolated early embryonic promoter/enhancer sequences from medfly to regulate the expression of tTA led to the production of a medfly strain that showed repressible embryonic lethality (Schetelig *et al.*, 2009).

***Transgenic sexing strains in fruit flies*** GSS in medfly are widely regarded as masterpieces of classical genetics, but they come with disadvantages – genetic instability, loss of progeny due to aneuploidy, difficulty of transferring to new species – which might be addressed by transgenic technology.

Technologies offering radiation replacement through bi-sex lethality as embryos or larvae would ideally require combination with one that allowed sexing for male-only release, at least with medfly and other tephritid species. A simple transgene-based enhancement to genetic sexing would be to insert a selectable marker on the Y chromosome. Depending on the nature of the marker, this would provide much the same properties as the classical translocation strains described above, but without the need for chromosome rearrangement. The Y chromosome of medfly is largely hetero-

chromatin, which might affect the expression of an inserted transgene, however Condon *et al*. (2007a) were able to establish proof-of-principle for genetic sexing by this method using a fluorescent marker.

An alternative approach would be to engineer a conditional female-specific lethal system. This would presumably be autosomal, though could in principle be located on the X chromosome. The first proof-of-principle experiments demonstrating such a system were carried out in D. melanogaster as repressible, female-specific lethal systems (Heinrich and Scott, 2000; Thomas *et al*., 2000). These used a two-part tetracycline-repressible system (Figure 4): yolk-protein promoters to regulate expression of the lethal gene, together with the Tet-off system described above to repress female lethality. These papers therefore illustrated a possible alternative means of generating sexing strains, or transgenic sexing strains (TSS), in pest species. The promoters used, however, act later in development than is desirable and their female-specificity may not be conserved between species (Tortiglione and Bownes, 1997).

This work was developed to produce TSS in pest species. In the medfly, Fu *et al*. (2007) used a sex-specific alternative splicing sequence from the C. capitata sex-determination gene, *transformer* (*Cctra*). From the endogenous *Cctra* gene, functional Tra protein is only expressed in females as the coding sequence is interrupted by exons in the males. Fu and colleagues took advantage of this female-specific excision to prevent expression of tTAV in males, and in females tTAV expression is suppressed by tetracycline using the Tet-off system. As with the bi-sex lethal medfly strains generated by the same group (Gong *et al*., 2005), described above, these strains are reared happily on larval diet containing tetracycline. When tetracycline is withheld, female larvae die and a male-only generation can be reared for release.

A major advantage of TSS over GSS is that the former technology can be more easily transferred to other species: very similar DNA constructs to that Fu *et al*. used to make medfly TSS have since been used for the development of TSS in the Mexican fruit fly (*Anastrepha ludens* Loew) (Koukidou *et al*., 2008). Use of the *tra* sex-alternate splicing has also been proposed for TSS in *L. cuprina (*Scott *et al*., 2004; Concha and Scott, 2009), and might be expected to transfer relatively easily to other Diptera without significant modification.

***Combining genetic sexing and radiation replacement*** It has been suggested that female-lethal TSS should be released without sterilising by radiation (Schliekelman and Gould, 2000; Thomas *et al*., 2000). In providing genetic sexing in the factory and conferring female lethality in progeny in the wild, female-specific RIDL can be regarded as offering genetic sexing and possible radiation replacement with one construct. Theoretical models suggest that, even disregarding the potential performance impact caused by irradiation, release of unirradiated TSS males homozygous for a female-lethal transgene would be similarly effective to SIT (Thomas *et al*., 2000). TSS in the mosquito *Ae. aegypti*, in which flightlessness in females (and consequent loss of viability) is repressed by tetracycline, have been developed for just such a purpose (Fu *et al*., 2010). The effect would be enhanced if the transgene were present in multiple copies, i.e. at several loci, but this will be counteracted by the increased fitness burden imposed by adding more copies (Schliekelman and Gould, 2000).

An additional potential benefit of releasing unirradiated TSS is that it may contribute to resistance management (e.g. for *Bt* toxin-expressing GM crops) in the target pest species (Alphey *et al*., 2007; Alphey *et al*., 2009). Survival of male progeny from such releases would allow for introgression of susceptibility alleles into the wild population, offering synergy with the IPM approach used against most pest insects.

The male adults of one such medfly TSS have been assessed for mating competitiveness in field cages (Morrison *et al*., 2009), and showed comparable performance to a wild-type strain and a GSS, VIENNA-8. Such preliminary tests need to be followed by assessments for field performance of this and similar strains to demonstrate their potential for RIDL/SIT. Detailed assessment of the productivity of TSS under mass rearing conditions must also be undertaken, as this is critical for SIT feasibility.

The potential for female-lethal TSS to produce crop-damaging male progeny, even in numbers limited by wild females present in the wild, may raise grower concerns about releasing such insects, though similar issues arise with $F_1$ sterility. Two possible solutions may be available through transgenesis, by making the TSS males effectively sterile. The testes-specific expression of fluorescent proteins achieved in medfly may be modified to express lethal effector genes in sperm (Scolari *et al*., 2008). This has been achieved in a mosquito (Windbichler *et al*., 2008). This approach might be compromised if the sperm load transferred by such males does not prevent the female from seeking further mates. Alternatively, the embryo-lethal phenotype described in medfly by Schetelig *et al*. (2009) could be used. By combining the functions of a TSS and these 'male-sterile' phenotypes, no progeny would result from mating between released and wild insects.

**Ceratitis capitata*: a case for genetic sexing by sexual transformation*** As described above, the medfly *tsl* GSS that are presently successfully used for massive production of male-only progeny are based on elimination of XX individuals as embryos by heat shock (Cáceres, 2002). Currently, more than 1.4 billion sterile male-only pupae are produced by strains per week in different facilities around the world. Hence, around 700 million XX female individuals are discarded as embryos in the release colony generation. An alternative strategy to female elimination could be their sexual transformation into XX males, which - following sterilisation - could be theoretically useful for SIT, together with XY males. Somewhat analogous to female-specific RIDL, it has also been suggested that a transgene inducing female-to-male sexual transformation could be used as a control agent without additional sterilisation (Schliekelman *et al*., 2005).

Sexual transformation of one sex into the other can be a natural or artificially induced phenomenon, observed in different metazoan species, in both vertebrates and invertebrates. Female-to-male (protogyny) or male-to-female (protandry) socially controlled sex changes have been described in two coral reef fish species (Fishelson, 1970; Robertson, 1972). Exposure to environmental pollutants such as the herbicide atrazine or the beef cattle growth promoter trenbolone can, firstly, completely feminise amphibian males (which can produce even viable eggs) (Hayes *et al*., 2010) and, secondly, irreversibly masculinise zebrafish (Morthorst *et al*., 2007). Embryonic male-killing by *Wolbachia* infection leads to female-only progeny in different dipteran species (Sheeley and McAllister, 2009). In *D. melanogaster*, which share a 120 mya common ancestry with Tephritidae fruitflies such as medfly, genetic mutations are known to cause either sexual transformation or sex-specific lethality (Cline and Meyer, 1996). Different mutant alleles in *Drosophila* of *Sex-lethal* (*Sxl*) – in *Drosophila* a master gene for female sex determination - lead either to male-only or female-only progeny, because of an improper dosage compensation of the X-linked genes controlled by *Sxl* (Salz and Erickson, 2010). Mutations in the *Drosophila transformer* (*tra*) and *transformer-2* (*tra-2*) genes lead to male-only progeny, comprising sterile XX pseudomales and fertile normal XY males (in *Drosophila*, the Y chromosome is required for male fertility, Sturtevant, 1945; Watanabe, 1975; Baker and Ridge, 1980; Ota *et al*., 1981).

It is not only spontaneous or induced mutations that can be used to provoke masculinisation of XX *Drosophila* flies. Fortier and Belote (2000) developed *Drosophila* transgenic strains producing partially masculinised XX individuals using transgene-mediated RNAi against tra-2.

Though the sex determination pathway is less well understood in other insects than in *Drosophila*, a few genetic studies have shed light on genetic control of maleness and femaleness in medfly and other insect species which could be useful for development of a masculinisation-based sexing strategy. In medfly, while the *Sxl* orthologue lacks a conserved function in sex determination, *transformer* (*Cctra*$^{ep}$) and *transformer-2* (*Cctra-2*$^{aux\text{-}ep}$)-homologous genes are both necessary for female sex determination, and for its epigenetic maintenance during development (Pane *et al*., 2002; Salvemini *et al*., 2002). CcTRA and CcDSX$^{M}$ have been ectopically expressed in *Drosophila* transgenic flies, showing conservation of the ability to provoke almost complete feminisation of XY and partial masculinisation of XX individuals, respectively (Saccone *et al*., 2007; Pane *et al*., 2005). As with *tra* in *Drosophila, Cctraep* uses alternative splicing to produce several transcripts from a single primary transcript. One of these alternative splicing forms is only found in XX individuals; only this mRNA encodes functional Tra protein – the other splicing forms include additional exonic sequences which introduce stop codons and/or frameshifts (Belote *et al*., 1989; O'Neil and Belote, 1992; Pane *et al*., 2002).

Surprisingly, *Cctra*$^{ep}$ contains multiple copies of short sequence corresponding to the *tra*/*tra-2* binding element of *Drosophila*, strongly indicating the possibility of a novel autoregulatory mechanism (Pane *et al*., 2002). Indeed a *Cctra*$^{ep}$ genomic fragment corresponding to this regulated region fused in a transgene construct and introduced into *Drosophila* produce female-specifically spliced mRNAs, indicating that *Drosophila Tra* and *Tra-2* can recognise and properly utilise cis-acting regulatory elements of *Cctra*$^{ep}$ (Fu *et al*., 2007).

Injection of double-stranded RNA targeting *Cctra*$^{ep}$ led to male-only progeny composed of XY males and XX pseudomales (Pane *et al*., 2002). Similar results have been obtained using dsRNA targeting the *Cctra-2*$^{aux\text{-}ep}$ orthologues which are required for female-specific splicing of *Cctra*$^{ep}$ and of *Ccdsx* (Salvemini *et al*., 2009). Interestingly, the XX pseudomales are fertile and can perform apparently normal courtship behaviour leading to productive copulation (Pane *et al*., 2002) (Salvemini *et al*., 2009). Furthermore this presumably transient depletion of the maternally inherited and zygotically produced female-specific mRNAs by embryonic RNAi caused in XX individuals a stable shift in the *Cctra*$^{ep}$ and *Ccdsx* alternative splicing from the female to the male mode until the adulthood. *Cctra*$^{ep}$ is therefore required for female sex determination and for epigenetic memory of this sex choice (Pane *et al*., 2002). Other elements of the sex determination pathway remain to be determined, in particular the nature of the male-determining locus on the Y chromosome and the signalling pathway from this locus to *Cctra*. No primary male-determining gene has been isolated until now in any insect species, and the Y-linked M factor in *Ceratitis* remains elusive (Willhoeft and Franz, 1996). Of course, the availability of an M factor would be ideal to develop a masculinisation strategy for sexing. In the hymenopterans *Apis mellifera* L. (European honeybee) and *Nasonia vitripennis* Walker (a parasitoid wasp) it has recently been clarified how two primary signals seem to work for haplo/diplo mechanisms: in diploid *A. mellifera* the heteroallelic *csd* locus combination leads to the activation of the feminizer gene (*fem*), while in haploid individuals the homoallelic combination leads to maleness, because of the absence of *fem* activity (Gempe *et al*., 2009). In *N. vitripennis* diploid embryos develop into females because of a maternal contribution of *transformer* (*Nvtra*) transcript, in combination with specific zygotic *Nvtra* transcription. In unfertilised eggs, however, maternal imprinting prevents zygotic transcription of the maternally derived *Nvtra* allele (Verhulst *et al*., 2010). Interestingly *fem* and *Nvtra* are orthologues of the medfly *transformer* gene: as in medfly (Pane *et al*., 2002) they both encode for a female-specific SR rich protein, they both produce this protein by female-specific splicing mechanism, they both present stop-containing male-specific exons. They both share with *CcTra* amino acid sequences, which are absent in the DmTRA protein. Finally, as observed by Pane *et al*. for Cctra (2002), *fem* and *Nvtra* are also able to positively autoregulate and are very sensitive to transient RNAi. This supports the hypothesis of Pane *et al*. (2002) of a wide evolutionary conservation of medfly-style *transformer* autoregu-

lating master gene for female sex determination in insects. *Cctra*$^{ep}$ orthologues have now been identified from several Dipteran species, in addition to those described above (Lagos *et al*., 2007; Ruiz *et al*., 2007; Concha and Scott, 2009; Hediger *et al*., 2010). This opens up the possibility of a generalised biotech sexing approach, based upon sex transformation. A first medfly transgenic strain has been developed that produces male-only progeny by transgene-mediated *Cctra*$^{ep}$-specific RNAi (Saccone *et al*., 2007). The progeny is composed of almost male-only progeny (XX fertile males and XY fertile males) with some intersexual XX sterile escapers (1-5%). This was a prototype, proof-of-principle strain only – use of such transgene-based sexual transformation for sexing and mass production of male-only progeny would require considerable further investigation, for example competition experiments showing that sterilised XX males are also useful to induce a reduction in the infesting population.

***Lepidoptera transgenic sexing strains*** To generate TSS in Lepidoptera for male-only release, Marec *et al*. (2005) proposed the insertion of a repressible, dominant lethal transgene into the female-determining W chromosome (females are heterogametic). Using such an approach, females would be transgenic and males wild-type under permissive conditions. Reared under restrictive conditions (i.e. in the release generation), females would die and males, which are wild-type, would be the sole survivors. Such males would still, however, require sterilisation by irradiation and would lack a genetic marker. Generating lines carrying W chromosome-inserted transgenes may also prove challenging and laborious, especially as multiple strains are likely required to test a range of position effects on the transgene.

Female-only releases of Lepidoptera have also been postulated as a possible improvement of SIT (White *et al*., 1976; Van Steenwyk *et al*., 1979). Lepidopteran females emit plumes of male-attracting sex pheromone, and the release of large numbers of females may act to divert wild males from mating with their female counterparts. This might act in much the same way as pheromone disruption, in which sources of synthetic pheromone are used as a tool for integrated pest management of some pest species (reviewed by Cardé and Minks, 1995). Female-only release would require the development of male-lethal sexing strains. In addition, releasing females only is unproven for large-scale control of any insect pest and, without death at the embryo stage, might lead to an unacceptable rise in crop damage by larvae. In contrast, if only males are released the number of eggs laid in the wild, and the consequent possible larval damage to crops, is limited by the number of wild females in the area. If this number is low, one might expect the damage from surviving larvae to be insignificant.

***Alternatives where SIT is not applicable – genetics in biological control*** Several countries in North Africa recently approached the International Atomic Energy Agency with a request to develop an SIT approach for control of desert locust (DL), *Schistocerca gregaria* Forskål. After much consideration, most experts agreed that DL is not a suitable target for traditional SIT. The economic damage caused by DL justifies such a project, but the target populations are so dispersed as to make the logistics of delivery of sterile individuals exceptionally difficult.

The last two major outbreaks of DL alone cost an estimated US$300 million in 1986-1989 and US$400 million in 2003-2005 and drew criticism for the size of spray operations (USAID, http://www.usaid.gov/our_work/humanitarian_assistance/disaster_assistance/locust), which are indiscriminate, and the chemicals broad-spectrum and neurotoxic.

Existing DL control programmes monitor field populations and initiate treatments when nymphal hopper stages reach high enough numbers to constitute an immediate threat. Although chlorinated hydrocarbon insecticide use is no longer common, other broad-spectrum insecticides are being supplemented by increasing use of entomopathogenic fungus *Metarhizium anisopliae* in Africa and Asia and in Australia against the plague locust, Chortoicetes terminifera Walker. The fungal biopesticide is decidedly narrow-spectrum and a good fit in the Integrated Pest Management (IPM) approach.

However, the fungal biopesticides are generally slow acting. In efforts to increase speed of action, Wang and St Leger (2007) inserted a gene from the scorpion, *Androctonus australis*, into *M. anisopliae* that increased the speed of action, but also increased potency. The venom was not expressed until the fungal hyphae grew through the cuticle and encountered the haemolymph. The venom peptide itself is topic only to insects insects (Zlotkin *et al*., 2000). Initial responses of the insects tested were decreased mobility and reduced feeding compared to the non-recombinant versions.

Delivery is key to lack of side-effects. Biological control agents (including sterile male insects) seek out the pest target and deliver themselves. On the other hand, sprayed biopesticides with narrow spectra of activity represent a residue burden to the environment. Systemic insecticides delivered by drip irrigation drastically reduces access to non-target organisms and fit well with IPM concepts. Transgenic plants are essentially 100% delivery of toxins to the crop needing protection, which is close to the immunisation model of public vaccinations against disease agents. Ritter (2009) described how many of these newer methods are resonating with modern agriculture within the context of increasing pressure on agriculture itself (Kiers *et al*., 2008).

***Regulation and public opinion*** Aside from the technical aspects of engineering specific genetic traits, the major obstacle to widespread use of transgene-carrying insect strains in the field is that of regulatory acceptance and uncertainty over public perception of the technology. Considerable progress has been made in these areas. On the regulatory side, several initiatives are ongoing or completed (reviewed by Beech *et al*., 2009). Two prominent completed initiatives are the NAPPO Standard RSPM27 "Guidelines for Importation and Confined Field Release of Transgenic Arthropods

in NAPPO Member Countries" (NAPPO, 2007) and the Final Environmental Impact Statement (FEIS) and associated Record of Decision published in 2008 by the USDA (USDA, 2008; Rose, 2009).

Potential risks must be evaluated on a case by case basis taking into account, the genetic traits inserted, the receiving environment and the niche of the insect. The FEIS was conducted on both fruit flies and pink bollworm and concluded that it was "the environmentally preferable alternative" compared to the use of insecticides and the existing irradiation-based SIT programme. Recently two additional initiatives have also been concluded: The Cartagena Protocol Ad Hoc Expert Group on Risk Assessment and Risk Management, sub-working group report on Living Modified Mosquitoes and the European Food Safety Authority contracted report, prepared by a consortium comprising of the Austrian Environment Agency, the international Atomic Energy Agency and the University of Bern, on the Environmental Risk Assessment Criteria for Genetically Modified Insects. Both of these documents indicate that potential risks must be evaluated on a case by case basis. Potential risks could include: horizontal and vertical gene flow, increase in host range, effects on predators and prey, changes in vector competence, changes in the mobility, fitness and survival potential of the target insect.

Public reaction is harder to assess, particularly at this relatively early stage. The FEIS included opportunity for public comment, including five public meetings, with few negative comments received (USDA, 2008). So far, the prospect of using engineered insects in SIT-like strategies has not encountered much resistance (e.g. Pew Initiative on Food and Biotechnology, 2004), though it would be premature to conclude that this will remain the case if and when these methods start to be deployed on a large scale. There is a possibility that some of the hostility directed at GM crops will spill over to GM insects, even though there are many technical and societal/cultural differences. It is also possible that the use of self-limiting strategies such as sterile insects may be confused with some unrelated, invasive, self-spreading genetic strategies that have been proposed – though not yet developed to prototype stage – for control of human disease vectors such as mosquitoes (Alphey *et al*., 2002; Alphey, 2009). As for any new technology, ongoing two-way communication between developers and key stakeholders will be key to avoiding potential future misunderstandings and roadblocks. Nonetheless, at present there seem reasonable grounds for optimism about the prospects for enhanced SIT.

## SUMMARY

As illustrated here, SIT has been, and continues to be, a hotbed of genetic innovation. The benefits of genetic sexing strains have been illustrated by their global adoption for medfly SIT. At the same time, transgenic technology offers a much wider spectrum of advances in genetic tools for SIT, from heritable marking to alternative methods for sterilisation.

Assuming the remaining technical, regulatory and public perception hurdles can be overcome – and much progress has been made in each of these areas – it is likely that transgenic technology will follow classical genetics into widespread field use, enhancing the efficiency of SIT and, maybe more importantly, increase the range of pest species that can be targeted by this environmentally friendly, species-specific method of control.

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