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Insect transgenesis and its potential role in agriculture and human health

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Abstract

The ability to genetically engineer insects other than *Drosophila melanogaster* has further extended modern genetic techniques into important insect pest species ranging from fruit fly pests of horticulture to mosquito vectors of human disease. In only a relatively short period of time, a range of transgenes have been inserted into more than 10 insect pest species. Genetic transformation of these pest species has proven to be a very important laboratory tool in analyzing gene function and effects on phenotype however the full extension of this technology into the field is yet to be realized. Here we briefly review the development of transgenic technology in pest insect species and discuss the challenges that remain in this applied area of insect genetics and entomology.

Keywords: Insect transgenesis; Transposable element; Insect genetic control

1. Introduction

The sterile insect technique (SIT) is an increasingly important component of integrated pest management programs for key agricultural pests (Tan, 2000; Wyss, 2000; Robinson, 2002). Operational use of the SIT continues to reveal areas where technology can still improve efficiency and thus lead to more cost effective programs. The transfer of genetic sexing technology to medfly, *Ceratitis capitata*, SIT programs is a recent example of the use of improved technology (Robinson et al., 1999). The International Atomic Energy Agency (IAEA) and the Food and Agriculture Organisation of the United Nations (FAO) continue to play a leading role in the development and implementation of this technology through their Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and the FAO/IAEA Agriculture and Biotechnology Laboratory at Seibersdorf, Austria.

The past 10 years have seen an explosion in the use of molecular biology in all biological sciences; especially in the fields of medicine and agriculture and particular emphasis has been placed on gene transfer technology. The recognition that the development of gene transfer techniques in pest insects could lead to improvements in the SIT, encouraged the agency to support and co-ordinate activities in this field. The major mechanism used to achieve this objective was through co-ordinated research projects (CRP). These projects are organized using a network of research teams who collaborate for a period of 5–6 years in achieving the common goal. Two CRPs have been completed and the results of the first were published in 1998 (IAEA, 1998) and this issue of IBMB summarizes the results of the second CRP, "Transgenic Technology and its Application in the SIT". It is encouraging that with the completion of the second CRP, transformation of many different insect pest species has been demonstrated and with some species the process is now rather routine.

The agency can take considerable credit for the successful development of gene transfer techniques in pest insects. Scientific progress in the field is now such that transgenic technology in pest insects can be moved from the laboratory to initial evaluations of strains

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under operational conditions of large scale rearing and in contained field cage situations to assess mating competitiveness of the transgenic strains. During the implementation of the CRPs, highly efficient vectors for pest insect transformation have been developed and widely applicable transformation markers have been identified. These developments have made the screening of putative transgenic individuals in any pest species extremely efficient and have led to the creation of transgenic strains in more than 10 pest insect species. In many pest insects molecular analyses of important gene systems have produced useful biological reagents for the development of transgenic strains for use in SIT and other control strategies.

2. Early developments

The development of gene cloning and *in vitro* gene splicing techniques in bacteria in the early 1970s suggested new possibilities for the use of genetic engineering in many fields of pure and applied biology. In the field of applied biology, important constraints in agricultural production and the many issues related to human health were considered to be targets for this new technology. In both these areas, insects, either as pests on agricultural crops or as vectors of disease, still represent a major problem for modern society. This present situation exists despite the best attempts of entomologists, chemists and ecologists to devise methods of insect control that effectively address a specific insect problem without generating new concerns. It is still the case that most insect problems are addressed by the application of chemical toxins, delivered either directly or, more recently for agricultural pests, through transgenic plants. In the public health area, insect-transmitted diseases are becoming an ever-growing problem for an increasing proportion of the world's population.

The application of genetic engineering to help solve insect-related problems was first discussed during a meeting sponsored by the Rockefeller Foundation in 1979 (Levin, 1979). At that time the possibilities of introducing specific genes into the genomes of pest insects seemed remote although the author did suggest that "in the more distant future it may be possible to use genetic engineering techniques to shift the food preferences of pest insects from crop plants to agriculturally noxious weeds".

In 1982, Rubin and Spradling (1982) were successful in transforming *Drosophila melanogaster* and this stimulated the elaboration of new ideas related to the use of this new technology in pest control (Cockburn et al., 1984; Beckendorf and Hoy, 1985). Most of the early attempts to transform pest insects were carried out using P-element based vectors that had been so successful with *Drosophila*. These attempts all failed and it was soon recognized that the P-element system depended on the presence of several host factors specific to this species or closely related ones (O'Brochta and Atkinson, 2000). Another major problem encountered in these early experiments was the lack of efficient transformation markers and reliance on antibiotic selection to identify putative transformants that proved to be highly ineffective. These failed attempts pointed towards the need for the identification of other transposable elements with a wider host range and this search has been very successful, as has the isolation of generic transformation markers (see below).

3. Transgenic technology: the current state of the art

Genetic transformation of non-drosophilid insects is now possible using four transposon-based gene vectors (for reviews see Handler, 2001, 2002; Atkinson, 2002; Atkinson et al., 2001). These vector systems, constructed from the *mariner*, *Minos, Hermes* and *piggyBac* transposable elements, have been used to transform 15 species of insects including Diptera, Hymenoptera, Lepidoptera and Coleoptera (see Table 1). These vectors are likely to be useful in a very wide range of insect species. Robust genetic marker systems for recognizing transgenic insects based on a variety of fluorescent proteins are now available (Horn et al., 2002). These dominant marker systems, combined with highly conserved gene regulatory sequences, provide reliable methods for detecting, maintaining and recognizing transgenic insects. The availability of these vector and marker systems has allowed numerous genetic manipulations in insects with agricultural and human health significance. One agricultural pest, the pink bollworm, has already been registered for testing a fluorescent marking system in a contained release, (Peloquin, personal communication) and a malaria vector, *Anopheles stephensi*, has been made partially refractory to an infectious pathogen (Ito et al., 2002). Several types of genetic manipulation have been proposed that may improve SIT programs and provide new methods for biological control (Robinson and Franz, 2000). In *Drosophila*, transgenic strains carrying constructs of relevance to improving the SIT have already been tested successfully, e.g. conditional gene expression systems have been used to achieve female-lethality for genetic-sexing, (Thomas et al., 2000; Heinrich and Scott, 2000; Horn and Wimmer, 2003). Models using other conditional lethal systems have been developed, e.g. the use of temperature sensitive mutations (Schliekelman and Gould, 2000a).

Although existing transposon vectors have proved to be widely useful, experimental evidence indicates that

Table 1
Successful germ-line transformation in pests insects

Common name	Family or superfamily	Species transformed	Element	References
Fruit flies	Tephritidae	*Ceratitis capitata*	*Minos*	Loukeris et al. (1995)
		Ceratitis capitata	*piggyBac*	Handler et al. (1998)
		Ceratitis capitata	*Hermes*	Michel et al. (2001)
		Bactrocera dorsalis	*piggyBac*	Handler and McCombs (2000)
		Anastrepha suspensa	*piggyBac*	Handler and Harrell (2001)
Houseflies	Muscidae	*Musca domestica*	*piggyBac*	Hediger et al. (2001)
		Musca domestica	*Mos*1	Yoshiyama et al. (2000)
		Stomoxys calcitrans	*Hermes*	O'Brochta et al. (2000)
Blowflies	Calliphoridae	*Lucilia cuprina*	*piggyBac*	Heinrich et al. (2002)
Mosquitoes	Culicidae	*Aedes aegypti*	*Hermes*	Jasinskiene et al. (1998)
		Aedes aegypti	*Mos*1	Coates et al. (1998)
		Aedes aegypti	*piggyBac*	Lobo et al. (2002)
		Anopheles gambiae	*piggyBac*	Grossman et al. (2001)
		Anopheles stephensi	*Minos*	Catteruccia et al. (2000)
		Anopheles stephensi	*piggyBac*	Ito et al. (2002)
		Anopheles albimanus	*piggyBac*	Perera et al. (2002)
		Culex quinquefasciatus	*Hermes*	Allen et al. (2001)
Sawflies	Hymenoptera	*Athalia rosae*	*piggyBac*	Sumitani et al. (2003)
Silkworm	Bombycidae	*Bombyx mori*	*piggybac*	Tamura et al. (2000)
Gelechiid moths	Gelechiidae	*Pectinophora gossypiella*	*piggyBac*	Peloquin et al. (2000)
Darkling beetles	Tenebrionidae	*Tribolium castaneum*	*piggyBac*	Berghammer et al. (1999)
		Tribolium castaneum	*Hermes*	Berghammer et al. (1999)

the transposable elements have a wide distribution and host range. This raises concerns about vector stability in transgenic strains when used in large scale rearing and for field release trials. Currently, limited information is available about mobile element interaction with the host genome and their ability to move between species. Safe and efficient implementation of transgenic systems to a broad variety of insect pests will require methods to ensure vector stability and strain integrity, while maintaining consistent levels of transgene expression. Heterologous recombination systems, tested in *Drosophila*, will allow vector immobilization after primary genomic integration events, and DNA insulator cassettes should minimize chromosomal effects on transgene expression levels.

4. Major areas for application of transgenic technology

4.1. Sterile insect technique

There are three key areas in SIT programmes where the use of transgenic strains can be of considerable importance. First, the development of a genetic marker would remove the need to mark released flies with fluorescent dust. The current process using fluorescent dye is inefficient, expensive and has a negative impact on the sterile insects and on the health of workers in a mass rearing facility. Secondly, transgenic approaches to the production of male only progeny could improve the efficiency of current systems and facilitate the expansion of the system to other pest species. Thirdly, the use of molecular methods to sterilize natural populations has been suggested (Alphey, 2002), but there are serious concerns as to the practicability of this approach. Any transgenic strain for use in the SIT will need to fulfill a minimum set of quality control parameters during mass rearing in order to maintain strain integrity and be suitable for economic mass rearing. This may require the development of specific rearing systems as was developed for genetic sexing strains based on classical genetics (Fisher and Caceres, 2000). It is considered very likely that the SIT will provide the first opportunity to use transgenic insects in any sort of field application, as there will be no vertical transmission of the transgene.

4.1.1. Mass rearing and strain stability

The SIT is based on the large-scale production of good quality insects for sterilization and release. The mass rearing process to produce the insects is on an industrial scale and requires that any special genetic strain, developed either using classical genetics of transgenesis, must be robust enough to maintain its integrity during long-term rearing under these conditions (Franz, 2002). The two associated factors modulating strain stability are numbers of insects reared and selective pressure. The high numbers of insects reared on a continuous basis provides the opportunity for extremely rare genetic/molecular events to occur and the highly selective rearing conditions will ensure that any new variant with even a small net gain in fitness

will be rapidly selected. This type of random process can rapidly destroy the specific characteristic of the strain for which it was developed. There is currently no data available as to how any transgenic strains will respond to this type of rearing and it is certainly possible that each strain will respond differently once exposed to this situation. Unfortunately the process cannot be simulated in a typical laboratory and conclusions about the usefulness of a specific strain can only be obtained a posteriori, a very expensive and long-term process. Assuming that molecular stability can be managed then the overall productivity of the strain in the facility becomes an issue. Strains that are debilitated in any way will present problems for the programme and will probably not be useful. The transfer of any strain from the laboratory environment to the real world of operational SIT is an unpredictable but essential process.

4.1.2. *Molecular vs. radiation induced sterility*

Radiation is now the method of choice for sterilizing insects for release in an SIT programme but there are now several experimental molecular systems that have been tested in *Drosophila* (Thomas et al., 2000; Heinrich and Scott, 2000). The motivation for developing these strains is the commonly held perception that radiation is the major cause of reduced effectiveness of the released insects in the field (Alphey and Andreasen, 2002; Alphey, 2002). However, the final competitiveness of the insect in the field is due to the accumulation of many effects, both genetic and environmental, that occur during the implementation of a field release programme with radiation being only a minor concern. At the genetic level, the vast majority of insects go through a severe bottleneck during initial colonisation which leads to a drastic reduction in overall genetic heterogeneity. This together with the extremely harsh selection pressure inherent in any mass rearing system undoubtedly leads to a reduction in the field fitness of the insect (Cayol, 2000). This means that during the life of a program there will be a gradual and unavoidable reduction in fly quality. Furthermore, every cohort of flies that is destined for release, as well being radiated, has to be marked with a dye, allowed to emerge, chilled and transported to the field and then released from an aircraft. This is a fairly rigorous set of procedures and together they will certainly impact negatively on field fitness. Replacing radiation with molecular sterility can only address one of these issues.

If a small increase in fitness can be confirmed when radiation is replaced by molecular sterilization then it has to rigorously demonstrated that the sterilizing effect of the alternative is as reliable as radiation obviously is in inducing sterility in large heterogeneous field populations. There are very serious problems associated with molecular sterility on this issue. Molecular sterility, in a similar way to radiation induced sterility, is based on the expression of "dominant lethality" in the zygote produced following mating of the sterile male with the field female. However, there is a critical difference between the two systems that is based on the concept of redundancy and the fact that radiation induced sterility is chromosome based, whereas molecular sterility is gene based. Molecular sterility relies on the expression of a single, identical dominant lethal gene in all the zygotes fertilized by the released male. Any variation in the interaction of the paternal and maternal genomes that leads to a suppression of gene expression will ensure that normal development of the zygote proceeds. This new variant will immediately be selected in the field population and the sterilizing effect of the released males will rapidly disappear. It may be suggested that putting several different molecular constructs into a strain will solve the problem (Schliekelman and Gould, 2000b) but this increase in complexity may bring with it other concerns. The situation with radiation is quite different in that following radiation every male insect carries in its sperm, a unique collection of many chromosomal rearrangements that can each act independently to cause the death of a zygote and hence sterility in the fertilized females. It is impossible for the field population to develop any sort of resistance to this type of lethality induction exhibiting this level of redundancy.

5. Disease control through engineering refractoriness

A major focus of insect transformation is the generation of mosquitoes that are refractory to the transmission of pathogens. The primary disease target has been malaria, due to both its medical and economic impact on developing countries in Africa, South Asia and South and Central America, although vector-based strategies and techniques developed for control of malaria can, in principle, be applied to other mosquito-borne diseases, such as dengue, and the wide range of viral encephalitis they transmit. The impetus for developing refractory mosquitoes came from the observation that refractoriness, which occurs naturally in the field, was under genetic control and could be successfully selected in laboratory strains of *Anopheles gambiae* (Collins et al., 1996). These mosquitoes were found to have an increased ability to encapsulate *Plasmodium* protozoa as they emerged from the epithelial midgut into the hemocoel and attention was focused on the immune system of the mosquito as a source of genes that could potentially be used to introduce artificial refractoriness into transgenic mosquitoes. Since then, several different aspects of the innate immune system of

mosquitoes have been explored as sources for genes that could confer resistance to pathogen transmission. Essential to the success of these studies is identifying promoters that allow expression of the immune response gene at the time that the pathogen is present in the female mosquito. Thus the vitellogenin gene promoter, which is expressed in response to a blood meal and promoters from gene expressed in the midgut have been used to drive the expression of immune response genes in transgenic mosquitoes (Kokoza et al., 2000). The expression profile of immune response genes in transgenic mosquitoes is important for two reasons. First it localizes expression of the transgene to the tissues encountered by the pathogen at a time when only the pathogen is likely to be present (after a blood meal in the case of female mosquitoes). Second, by confining expression of the transgene to these tissues, at a defined time, any negative effects that transgene expression might have on fitness will, in theory, be reduced.

The first practical demonstration that a gene introduced into a mosquito could result in a decrease in the ability of the mosquito to transmit a pathogen was, however, achieved when a Sindbis virus containing the antisense gene for the dengue virus coat protein was found to lead to a marked decrease in the presence of dengue viral particles in infected *Aedes aegypti* mosquitoes (Olson et al., 1996). These mosquitoes were not transgenic and so the ability of the transgene present in the genetically engineered virus to reduce dengue virus transmission in subsequent generations could not be determined. The development of transposable element-mediated insect transgenesis systems for mosquitoes led to the generation of mosquitoes that contained some of the chimeric immune response genes described above, although as yet most effector genes remain untested in transgenic mosquitoes, due in part to both the small number of laboratories in which mosquito transgenesis is performed and the technical difficulty of this technique. The *defensin* gene from *Ae. aegypti*, placed under the control of the vitellogenin promoter from this species, was re-introduced into *Ae. aegypti* using the *Hermes* transposable element (Kokoza et al., 2000). The mid-gut specific carboxypeptidase promoter has been used to drive expression of the SM1 synthetic polypeptide in transgenic lines of *Anopheles stephensi* (Ito et al., 2002). This synthetic small peptide binds to the midgut and salivary glands and blocks transmission of *Plasmodium berghei*, the pathogen of rodent malaria, in these transgenic lines. When the same promoter was used to drive the expression of bee venom phospholipase, which inhibits oocyst formation in the blood meal, a reduction of *P. bergei* was observed in transgenic lines of *An. stephensi* (Moreira et al., 2000). Attacking *Plasmodium* in the midgut, or at the midgut/hemocoel boundary, has been a favored site of transgene expression since it is in these tissues that the numbers of the parasite are at their lowest and so represent perhaps its most vulnerable stage in the mosquito. Strategies aimed at expressing transgenes in the salivary glands of mosquitoes are also under development and, at least using viral-based expression systems, a single chain antibody targeted to the circumsporozoite surface protein led to a significant reduction in the number of *P. gallinaceum* sporozooites in *Ae. aegypti* infected with the transgenic virus (Capurro et al., 2000). Transgenic mosquitoes containing the corresponding transgene placed in a transposable element are yet to be tested for their ability to prevent transmission of the parasite.

The publication of the *An. gambiae* genome sequence and subsequent analysis by homology-based search programs identified a total of 242 genes from 18 gene families that are potentially involved in the immune response in this mosquito (Christophides et al., 2002). The use of functional genomics technologies, such as microchip analysis, will identify which of these are transcriptionally regulated in response to challenge by parasite infection and, inevitably, many will be tested for their ability to confer refractoriness on transgenic mosquitoes. The immune response is only one source of genes that could be engineered to prevent the transmission of pathogens through female mosquitoes following the uptake of a blood meal. Other types of genes, such as those that encode peptides that reduce or eliminate the ability of the pathogen to bind to cell surface receptors present on midgut cell, hemocytes or salivary gland cells, or those that can be modified to directly attack the parasite, would also be predicted to be uncovered using the genomic tools now available for this important mosquito species.

Within the confines of the laboratory and the insectary, the development of mosquitoes genetically altered to be refractory to the transmission of *Plasmodium* species remains the most elegant and advanced use of transgenesis in non-drosophilid insects. As a laboratory tool, transposable element mediated transgenesis is thus proving to be a key technology. There are, however, still limitations to this technology that must be overcome and, as mentioned above, even with the modest number of effector genes now available for testing in mosquitoes, the ability to routinely transform mosquitoes still remains a significant impediment with transformation frequencies only in the order of 10% in two species of mosquito, *Ae. aegypti* and *An. stephensi*. *Ae. aegypti*, the significant vector of yellow fever and dengue, is not a vector of human malaria but is used as a model for its human counterpart because it does transmit avian malaria and because its eggs are easy to microinject and handle in the laboratory. *An. stephensi* is a vector of both human and rodent malaria and is also favored in the laboratory due to the relative ease

with which its eggs can be handled and microinjected. *An. gambiae*, the major vector of human malaria, has been genetically transformed using the *piggyBac* transposable element, however transformation frequencies are not high, due in part to the difficulty in handling these embryos. This remains a significant reason why transgenic technology has yet to be widely adopted for this critical species. Mosquito transformation vectors that lead to high rates of transformation of this species still need to be developed if the full power of transgenic technology is to be successfully applied to this key vector of human malaria.

6. Regulatory issues

From both practical and regulatory standpoints, there are two broad strategies for the release of transgenic insects. One involves the use of the SIT in which the genetically engineered strain is sterilized (typically by irradiation) prior to release. An inundative release of these insects ensues, and the populations of the pest in the field are reduced and then eliminated.. Since the genetically engineered strain is sterile, there is no vertical transmission of the transgene to the field population. There remains however the rare, and essentially uncharacterized events related to horizontal transfer. For SIT to be successful, genetically engineered, sterilized insects must be able to successfully find and mate with individuals of the wild-type, target population. Given that irradiation leads to a small decrease in mating competitiveness, it is important that any genetic engineering performed on these release strains does not further compromise their ability to survive and mate in the field. The fact that sterilization renders these insects incapable of passing their genes on to successive generations, combined with the high levels of quality control required for the production of sterile insects in SIT programs, means that it is likely that it is through SIT programs that genetically engineered pest insect strains will make their first appearance in the field.

Genetic alternatives that result in sterilization without irradiation have been proposed, and have shown promise in small studies in the *D. melanogaster* (Thomas et al., 2000; Heinrich and Scott, 2000). As yet none of this type of technology has been transferred to pest insects. To be successful in the field, these genes and promoters must function correctly in the target insect pest species and any genetic leakiness must be eliminated if these genetic strategies are to compete with radiation-based sterilization techniques. Furthermore, regulatory agencies will most likely demand that these genetic systems be stable so that the desired level of genetic death can be predicted and delivered to the population over the course of the pest control program. These are tall orders for a nascent technology, but may be achievable.

The spread of beneficial genes through target populations presents the greatest regulatory challenge for the use of transgenic insects. The technology, if it is to be successful, is based on the ability of the vector associated with the transgene to spread through the target pest population for many generations following the release of the transgenic strain. The strategy thus requires that any negative fitness costs arising from transgenesis be small enough not to compromise gene spreading. It also requires complete linkage of the transgene and the vector and stable and reliable expression of the transgene through time and space. Thus, far from the transgenic system being contained, it is designed to spread, and to do so quickly, so that the transmission of the target human disease can be decreased as soon as possible following release. The design of this system thus places it at odds with other transgenic technologies that are designed to be contained, either for regulatory or commercial reasons, or both.

A recent report by the United States National Research Council examined the risks arising from the development of transgenic technology in animals (National Research Council, 2002). Based on the likelihood of transgenic animals to become feral, their likelihood of escape from containment, their mobility and the likelihood of their being unforeseen effects on ecosystems, they ranked transgenic insects and transgenic fish as being those animals in which transgenesis carried with it the greatest risks. Yet, as we have discussed, strategies that seek to generate transgenic mosquitoes that spread beneficial transgenes through populations actually capitalize on many of these properties. We wish them to be able to survive and reproduce in the wild and we aim to have both the insects and their transgenes disperse through a population.

Does this mean that those that develop transgenic insects for beneficial medical and agricultural purposes and those who oppose transgenesis are likely to make transgenic insects the next battlefield for the ongoing debate about the application of biotechnology for human welfare? Clearly there are many unknowns to be addressed, yet many of these are measurable using existing technologies. The cost of transgenesis on the fitness of transgenic insects can be measured using a range of life table parameters. The ability of transposable elements to transpose in insect species into which they have been introduced can be measured, and data for the behavior of *Hermes*, *mariner* and *piggyBac* in transgenic lines of *Ae. aegypti* now exists (O'Brochta et al., 2003). Factors that influence transposable element behavior in insects can also be measured and host proteins that interact with these elements ident-

ified. Thus experimental data increasing our ability to predict the behavior of transgenic insects can be gathered and the examination of these data will be critical in developing protocols for the release of transgenic insects, be they for SIT programs or for programs in which the aim is to spread a beneficial gene through a pest insect population. If it can be demonstrated, through experiment and trial, that insect strains can be successfully developed that do prevent the spread of insect-borne human disease, then the argument can be advanced that these technologies are no different to a therapeutic recombinant-derived drug or vaccine, except that they do not need to be introduced into humans. As such, given the severity and prevalence of insect-borne human disease, the argument may well become why these new technologies should not be applied to the betterment of human welfare.

References

Allen, M.L., O'Brochta, D.A., Atkinson, P.W., Levesque, C.S., 2001. Stable, germ-line transformation of *Culex quinquefasciatus* (Diptera: Culicinae). J. Med. Entomol. 38, 701–710.

Alphey, L., 2002. Re-engineering the sterile insect technique. Insect Biochem. Mol. Biol. 32, 1243–1247.

Alphey, L., Andreasen, M., 2002. Dominant lethality and insect population control. Molec. Biochem. Parasit. 121, 173–178.

Atkinson, P.W., 2002. Genetic engineering in insects of agricultural importance. Insect Biochem. Mol. Biol. 32, 1237–1242.

Atkinson, P.W., Pinkerton, A.C., O'Brochta, D.A., 2001. Genetic transformation systems in insects. Annu. Rev. Entomol. 46, 317–346.

Beckendorf, S.K., Hoy, M.A., 1985. Genetic improvement of arthropod natural enemies through selection, hybridization or genetic engineering techniques. Biological Control in Agricultural IPM Systems. Academic Press, Inc, pp. 167–184.

Berghammer, A.J., Klingler, M., Wimmer, E.A., 1999. A universal marker for transgenic insects. Nature 402, 370–371.

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

Cayol, J.P., 2000. Changes in sexual behaviour and life history traits of tephritid species caused by mass rearing processes. In: Aluha, M., Norrbom, A.L. (Eds.), Fruit Flies: Phylogeny and Evolution of Behaviour. CRC Press, Boca Raton.

Christophides, G.K., Zdobnov, E., Barillas-Mury, C., Birney, E., Blandin, S., Blass, C., Brey, P.T., Collins, F.H., Danielli, A., Dimopoulos, G., Hetru, C., Hoa, N.T., Hoffmann, J.A., Kanzok, S.M., Letunic, I., Levashina, E.A., Loukeris, T.G., Lycett, G., Meister, S., Michel, K., Moita, L.F., Muller, H.M., Osta, M.A., Paskewitz, S.M., Reichhart, J.M., Rzhetsky, A., Troxler, L., Vernick, K.D., Vlachou, D., Volz, J., von Mering, C., Xu, J., Zheng, L., Bork, P., Kafatos, F.C., 2002. Immunity-related genes and gene families in *Anopheles gambiae*. Science 298, 159–165.

Coates, C.J., Jasinskiene, N., Miyashiro, L., James, A.A., 1998. *Mariner* transposition and transformation of the yellow fever mosquito, *Aedes aegypti*. Proc. Natl. Acad. Sci. USA 95, 3742–3751.

Cockburn, A.F., Howells, A.J., Whitten, M.J., 1984. Recombinant DNA technology and genetic control of pest insects. Biotech. Gen. Eng. Rev. 2, 69–92.

Collins, F.H., Sakai, R.K., Vernick, K.D., Paskewitz, S., Seeley, D.C., Miller, L.H., Collins, W.E., Campell, C.C., Gwadz, R.W., 1996. Genetic selection of a Plasmodium-refractory strain of the malaria vector *Anopheles gambiae*. Science 234, 607–610.

Capurro, M., Coleman, J., Beerntsen, B.T., Myles, K.M., Olsen, K.E., Rocha, E., Krettl, A.U., James, A.A., 2000. Virus-expressed, recombinant single-chain antibody blocks sporozoite infection of salivary glands in *Plasmodium gallinaceum*-infected *Aedes aegypti*. A. J. Trop. Med. Hyg. 62, 427–433.

Fisher, K., Caceres, C., 2000. A filter rearing system for mass reared genetic sexing strains of Mediterranean fruit fly (Diptera: Tephritidae). In: Tan, K.H. (Ed.), Area-Wide Management of Fruit Flies and Other Major Insect Pests. Universiti Sains Malaysia Press, Penang, Malaysia, pp. 543–550.

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, 73–84.

Grossman, G.L., Rafferty, C.S., Clayton, J.R., Stevens, T.K., Mukabayire, O., Benedict, M.Q., 2001. Germline transformation of the malaria vector, *Anopheles gambiae*, with the *piggyBac* transposable element. Insect Mol. Biol. 10, 597–604.

Handler, A.M., 2001. A current perspective on insect gene transfer. Insect Biochem. Mol. Biol. 31, 111–128.

Handler, A.M., 2002. Prospects for using genetic transformation for improved SIT and new biocontrol methods. Genetica 116, 137–149.

Handler, A.M., Harrell, R.A., 2001. Transformation of the Caribbean fruit fly with a *piggyBac* transposon vector marked with polyubiquitin-regulated GFP. Insect Biochem. Mol. Biol. 31, 199–205.

Handler, A.M., McCombs, S.D., 2000. The moth transposon, piggyBac, mediates germ-line transformation in the Oriental fruit fly and exists in its genome. Insect Mol. Biol 9, 605–612.

Handler, A.M., McCombs, S.D., Fraser, M.J., 1998. The lepidopteran transposon vector *piggyBac*, mediates germline transformation in the Mediterranean fruit fly. Proc. Natl. Acad. Sci. USA 95, 7520–7525.

Hediger, M., Niessen, M., Wimmer, E.A., Dübendorfer, A., Bopp, D., 2001. Genetic transformation of the housefly *Musca domestica* with the lepidopteran derived transposon *piggyBac*. Insect Mol. Biol. 10, 113–119.

Heinrich, J.C., Scott, M.J., 2000. A repressible female-specific lethal genetic system for making transgenic insect strains suitable for a sterile-release program. Proc. Natl. Acad. Sci. USA 97, 8229–8232.

Heinrich, J.C., Li, X., Henry, R.A., Haack, N., Stringfellow, L., Heath, A.C.G., Scott, M.J., 2002. Germ-line transformation of the Australian sheep blowfly *Lucilia cuprina*. Insect Mol. Biol. 11, 1–10.

Horn, C., Wimmer, E.A., 2003. A transgene-based, embryo-specfic lethality system for insect pest control management. Nat. Biotechnol. 21, 64–70.

Horn, C., Schmid, B.G.M., Pogoda, F.S., Wimmer, E.A., 2002. Fluorescent transformation markers for insect transgenesis. Insect Biochem. Mol. Biol. 32, 1221–1235.

IAEA, 1998. Genetic Engineering Technology for the Improvement of the Sterile Insect Technique. IAEA-TECDOC-993, 78 pp.

Ito, J., Ghosh, A., Moreira, L., Wimmer, E.A., Jacobs-Lorena, M., 2002. Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. Nature 417, 452–455.

Jasinskiene, N., Coates, C.J., Benedict, M.Q., Cornel, A.J., Rafferty, C.S., James, A.A., Collins, F.H., 1998. Stable, transposon mediated transformation of the yellow fever mosquito, *Aedes aegypti*, using the *Hermes* element from the housefly. Proc. Natl. Acad. Sci. USA 95, 3743–3747.

Kokoza, V., Ahmed, A., Chao, W.L., Jasinskiene, N., James, A.A., Raikhel, A.S., 2000. Engineering blood-meal activated systemic

immunity in the yellow fever mosquito, *Aedes aegypti*. Proc. Natl. Acad. Sci. USA 97, 9144–9149.

Levin, B.R., 1979. Problems and promise in genetic engineering in its potential applications to insect management. In: Hoy, M.A., McKelvey, J.J. (Eds.), Genetics in Relation to Insect Management. The Rockefeller Foundation, pp. 170–176.

Lobo, N.F., Hua-Van, A., Li, X., Nolen, B.M., Fraser, M.J., 2002. Germ line transformation of the yellow fever mosquito, *Aedes aegypti*, mediated by transpositional insertion of a *piggyBac* vector. Insect Mol. Biol. 11, 133–139.

Loukeris, T.G., Livadaras, I., Arca, B., Zabalou, S., Savakis, C., 1995. Gene transfer into the Medfly, *Ceratitis capitata*, with a *Drosophila hydei* transposable element. Science 270, 2002–2005.

Michel, K., Stamenova, A., Pinkerton, A.C., Franz, G., Robinson, A.S., Gariou-Papalexiou, A., Zacharopoulou, A., O'Brochta, D.A., Atkinson, P.W., 2001. Hermes-mediated germ-line transformation of the Mediterranean fruit fly *Ceratitis capitata*. Insect Mol. Biol. 10, 155–162.

Moreira, L.A., Edwards, M.J., Adhami, F., Jasinskiene, N., James, A.A., Jacobs-Lorena, M., 2000. Robust gut-specific gene expressio in transgenic *Aedes aegypti* mosquitoes. Proc. Natl. Acad. Sci. USA 97, 10895–10898.

National Research Council. 2002. Animal Biotechnology. Science-Based Concerns. The National Academies Press, Washington, DC, 181 pp.

O'Brochta, D.A., Atkinson, P.W., 2000. Transposable elements and gene transformation in non-Drosophilid insects. Insect Biochem. Molec. Biol. 26, 739–753.

O'Brochta, D.A., Atkinson, P.W., Lehane, M.J., 2000. Transformation of *Stomoxys calcitrans* with a Hermes gene vector. Insect Mol. Biol. 9, 531–538.

O'Brochta, D.A., Sethuraman, N., Wilson, R., Hice, R.H., Pinkerton, A.C., Levesque, C.S., Bideshi, D.K., Jasinskiene, N., Coates, C.J., James, A.A., Lehane, M.J., Atkinson, P.W., 2003. Gene vector and transposable element behavior in mosquitoes. J. Exp. Biol. 206, 3823–3834.

Olson, K.E., Higgs, S., Gaines, P.J., Powers, A.M., Davis, B.S., Kanrud, K.I., Carlson, J.O., Blair, C.D., Beaty, B.J., 1996. Genetically engineered resistance to dengue-2 virus transmission in mosquitoes. Science 272, 884–886.

Peloquin, J.J., Thibault, S., Staten, R., Miller, T.A., 2000. Germ-line transformation of pink bollworm (Lepidoptera: Gelechiidae) mediated by the piggyBac transposable element. Insect Mol. Biol. 9, 323–333.

Perera, O.P., Harrell, R.A., Handler, A.M., 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, 291–297.

Robinson, A.S., 2002. Mutations and their use in insect control. Mut. Res. 511, 113–132.

Robinson, A.S., Franz, G., 2000. The application of transgenic insect technology in the sterile insect technique. In: Handler, M., James, A.A. (Eds.), Insect Transgenesis:Methods and Applications. CRC Press, Baton Rouge, pp. 307–319.

Robinson, A.S., Franz, G., Fisher, K., 1999. Genetic sexing strains in the medfly, *Ceratitis capitata*: Development, mass rearing and field applications. Trends Entomol 2, 81–104.

Rubin, G.M., Spradling, A.C., 1982. Genetic transformation of Drosophila with transposable element vectors. Science 218, 348–353.

Schliekelman, P., Gould, F., 2000a. Pest control by the introduction of a conditional lethal trait on multiple loci: Potential, limitations and optimal strategies. J. Econ. Entomol. 93, 1543–1565.

Schliekelman, P., Gould, F., 2000b. Pest control by the release of insects carrying a female-killing allele on multiple loci. J. Econ. Entomol. 93, 1543–1565.

Sumitani, M., Yamamoto, D.S., Oishi, K., Lee, J.M., Hatakeyama, M., 2003. Germline transformation of the sawfly, *Athalia rosae* (Hymenoptera: Symphyta), mediated by a *piggyBac*-derived vector. Insect Biochem. Molec. Biol. 33, 449–458.

Tamura, T., Thibert, T., Royer, C., Kanda, T., Eappen, A., Kamba, M., Kômoto, N., Thomas, J.-L., Mauchamp, B., Chavancy, G., Shirk, P., Fraser, M., Prud'homme, J.-C., Couble, P., 2000. A *piggybac* element-derived vector efficiently promotes germ-line transformation in the silkworm *Bombyx mori* L. Nat. Biotechnol. 18, 81–84.

Tan, K.-H. (Ed.), 2000. Area-wide Control of Fruit Flies and other Insect Pests. Penerbit Universiti Sains, Malayasia.

Thomas, D.T., Donnelly, C.A., Wood, R.J., Alphey, L.S., 2000. Insect population control using a dominant, repressible, lethal genetic system. Science 287, 2474–2476.

Wyss, J.H., 2000. Screwworm eradication in the Americas: overview. In: Tan, K.-H. (Ed.), Area-wide Control of Fruit Flies and other Insect Pests. Penerbit Universiti Sains, Malayasia, pp. 79–87.

Yoshiyama, M., Honda, H., Kimura, K., 2000. Successful transformation of the housefly, *Musca domestica* (Diptera: Muscidae) with the transposable element, mariner. Appl. Entomol. Zool. 35, 321–325.