

GENETICALLY MODIFIED INSECTS – A REVIEW

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ABSTRACT: Genetically modified insects are those insects, which have been genetically modified for various reasons like agricultural production, oil production and pest management. The main purpose of producing the genetically modified insect is to manage the agricultural pest which are causing severe damage to the yield, quality, environment and human health and reducing the use of synthetic chemical pesticides. These insects are made transgenic when one or more DNA sequences from other organisms are inserted into their genome. Their genetic modification has been proposed as a new way of controlling insect pests. The advantages of GM insects outnumbers the disadvantages of this technology but sound research on the public safety and sustainable ecological balance is necessary so that technology might not overestimate the right of future generation in the matter of preservation of our ecology and self-sustained nature.

Key words : Genetically modified insects, DNA, transgenic, ecological balance.

INTRODUCTION

Genetically modified insects are those insects which have been genetically modified for various reasons like agricultural production, oil production and pest management. These insects are made transgenic when one or more DNA sequences from other organisms are inserted into their genome. Main purpose of producing the genetically modified insect is to manage the agricultural pest which are causing severe damage to the yield, quality, environment and human health and reducing the use of synthetic chemical pesticides. Genetically modified insects are being developed with a view to suppress insect populations of the insect species which spread human diseases such as malaria and dengue fever.

Genetic manipulations of insects and other arthropods are an important tool for the study of the molecular basis of development and the evolutionary process (Horn and Wimmer, 2000). The use of these methodologies is also being considered as a possible solution to certain medical and agricultural problems caused by some insects, through the cautious releasing in nature of insects with desirable genetic alterations (Irvin *et al*, 2004).

The use of genetically modified (GM) insects represents a novel and innovative tool to address the insect-borne disease of humans and crop pest losses. It is, however, likely that the risk perception of the public and the acceptability of such risks, when balanced against potential benefits, will ultimately decide the pace of development of GM insects (Camilla *et al*, 2012). Origin of Genetic engineering in insects, accomplishing genetic

transformation, heritable modifications, genetic systems, RIDL, strategies in field, OXITEC and regulatory initiatives are reviewed by Kiran Gandhi *et al* (2014).

Why GM insects?

- For area-wide control of pests
- As bioreactors to produce pharma products
- To develop virus-resistant lines
- To enhance agricultural production, productivity
- To benefit public health
- To improve disease resistance, pollination attributes in honey bees and high quality silk production in silk moth (Gopinathan, 1992).

History of GM insects

Prior to the 1950s, one of the biggest pests of cattle in the US was a critter called *Cochliomyia homnivorax*, the New World Screw-worm. Raymond Bushland and Edward Knipling hypothesized that if we could sterilize male flies, each male would prevent multiple females from laying eggs and eventually the population would drop wherein the sterile males would outnumber the fertile males. Experiment on sterile insect resistance management technique was carried out aiming high sterile to fertile ratio which was achieved by using gamma irradiation, UV rays and mutagens like ethyl methyl sulphinate. The concept of genetic control of insect pest was given by E. F. Knipling which marks the history of GM insects (Joe, 2010).

The germ-line transformation was first demonstrated more than 20 years ago in *Drosophila melanogaster* by

the use of the transposable *P* element (Rubin and Spradling, 1982). The *Drosophila* gene for wild type eye color, *rosy+*, was stably introduced into the genome of a *rosy* mutant strain by a *P* transposable element vector and the resulting red eye color was stably inherited in subsequent generations. However, studies in many different species showed that this element was non-functional outside the *Drosophilidae*. The *P*-element system is dependent on the presence of several host factors specific to this species, or to closely related species. This has restricted the *P* mediated transformation to a few species of subgenus *Sophophora* of the genus *Drosophila* (Handler *et al*, 1993). The first transposon-mediated transformation of a non-drosophilid insect was registered 13 years later in the Mediterranean fruit fly, *Ceratitidis capitata*, using the *minos*-mediated transformation (Loukeris *et al*, 1995).

Methods of genetic transfer

1. Physical Methods

Microinjection : Microinjection refers to the use of a glass micropipette to inject a liquid substance at a microscopic or borderline macroscopic level. The target is often a living cell but may also include intercellular space. It is a well established technique used routinely for injection of animal cells, tissues and embryos to insert genetic material such as DNA, RNA, proteins and macromolecules directly into animal cells or embryos, a cell cytoplasm or nucleus.

Biolistics : A gene gun or a biolistic particle delivery system, originally designed for plant transformation, is a device for delivering exogenous DNA (transgene) to cells. The payload is an elemental particle of a heavy metal coated with DNA, typically plasmid DNA. This technique is often simply referred as biolistics.

Lipofection : Also called as liposome transfection, it is a technique used to inject genetic material into a cell by means of liposomes, which are vesicles that can easily merge with the cell membrane since they are both made of a phospholipids bilayer. Lipofection principle is to associate nucleic acids with cationic lipid formulation.

Electroporation : It refers to the action or process of introducing DNA or chromosomes into bacteria or other cells using a pulse of electricity to open the pores in the cell membranes briefly. Also called as electroporation, it is a microbiology technique in which an electrical field is applied to cells in order to increase the permeability of the cell membrane, allowing chemicals, drugs or DNA to be introduced into the cell.

2. Biological Methods

Transposable elements: Transposons are mobile

pieces of DNA that do not remain fixed at one genomic location, move from one site on a chromosome to another (Liao, 2000). They are rare sequences of DNA that can move around to different positions within the genome of a single cell, a process called transposition. In the process, they can cause mutations and change the amount of DNA in the genome. Transposons are also called “jumping genes”, and are examples of mobile genetic elements. These were discovered by Barbara McClintock early in her career and the discovery earned her a Nobel prize in 1983. There are a variety of mobile genetic elements, and they can be grouped based on their mechanism of transposition.

- Class I mobile genetic elements, or retrotransposons, move in the genome by being transcribed to RNA and then back to DNA by reverse transcriptase.
- Class II mobile genetic elements move directly from one position to another within the genome using a transposase to “cut and paste” them within the genome.

Transposons make up a large fraction of genome sizes and are very useful to researchers as a means to alter DNA inside of a living organism. The commonly used transposons in insect transformation are *Minos*, *Hermes*, *Mos 1* (*mariner*) and *piggyBac*. The transposable element vector systems used in the transformation of insects genetically. Transposable element *Hermes* from host *Musca domestica* was used to genetically modify *Drosophila melanogaster*, *Aedes aegypti*, *Tribolium castaneum*, *Ceratitidis capitata*, *Culex quinquefasciatus* and *Stomoxys calcitrans* as reported by different authors. Similarly, the insects which were modified by using other transposable elements like *hobo* from *D. melanogaster*, *Mimos* from *D. hydei* etc have been reported.

To generate transgenic insects, the transgene of interest must be introduced into the germline. Transposable elements are commonly used as vectors for genomic integration in insects. The most promising transposons available at present are the *hAT* family element *Hermes*, the *mariner* element *Mos1*, the *Tc1/mariner* element *Minos* and the *TTAA*-specific element *piggyBac*. For the stable integration of a transgene construct, two separate plasmids are used. One plasmid-the donor carries the transgene of interest (orange) and a visibly detectable transformation marker (green) within functional terminal inverted repeats (TIRs). The second plasmid-the helper encodes the transposase enzyme (pink) but cannot itself transpose, because the flanking TIRs are missing or defective. In most successful

insect transgenesis approaches, the two plasmids are microinjected into the region of early (pre-cellularized) embryos that is thought to contain the germplasm. In this region, the primordial germ cells form and some of their nuclei will take up the plasmids. As the embryo develops into an adult, the expressed transposase will mediate the transposition of the transgene construct from the donor plasmid onto a chromosome. So, some of the germline cells will contain genomic transgene insertions. If such an insertion is transmitted to the offspring, the individual receiving the insertion is stably transformed. Because the efficiency of genomic transgene insertion is low, a reliable and easily detectable transformation marker is necessary to identify the transgenic individuals (Wimmer, 2003).

Markers for insect transgenesis

- ✓ Universal marker used for gene transfer in any species is Green Fluorescent Protein (GFP).
- ✓ It is obtained from the jelly fish *Aequorea victoria*
- ✓ Active in both animal and plant kingdom.
- ✓ It requires strong promoter.
- ✓ Causes cytotoxicity when expressed at high levels (Berghammer *et al*, 1999).

Recently, an interesting marker system was developed based on the properties of fluorescent proteins like GFP (green fluorescent protein) and their variants. The GFP gene from the jellyfish *Aequorea victoria* (Prasher *et al*, 1992) is well suited, since it is easily detectable *in vivo* and has proved to be functional in different tissues of many heterologous systems (Tsien, 1998). It has been used in several organisms as insects, plants, fish, mice and cells of mammals (Bagis and Keskinetepe, 2001). For the expression control of GFP several promoters have been used. Constitutive promoters active in all cells provide the advantage of allowing selection of transformants at all stages since GFP fluorescence can be scored in living embryos, larvae, and adults.

Eye colour genes as transformation markers

Eye color genes are quite often used as transformation markers in insects carrying eye color mutations allowing the identification of transformants by mutant-rescue selection. In these cases, the marker gene represents the wild-type allele of a gene that, when mutated, causes a recessive, visible but viable phenotype. However, the lack of suitable recipient mutant strains for most insects of medical and agricultural importance limits their application (Finokiet *et al*, 2007). Initially, the search for

dominant-acting selection markers that could act independently of pre-existing mutant strains focused on genes which confer chemical or drug resistance. These marker systems showed low reliability and efficiency (Handler, 2001; Horn *et al*, 2002).

How GM insects?

1. Direct genome modification

Direct genome modification is the insertion of desirable gene sequence or DNA into the insect species. This is of two type:

➤ Sterile insect technique (SIT)

Non-viable or infertile offspring is the ultimate goal of SIT; the sterile males inseminate the females, producing non-viable eggs, thus reducing the population.

➤ rDNA technology

After selection of a trait, researchers have two options for inserting the gene into the insect's genome. One is using transposons or "jumping genes," have the ability to cut and paste themselves into and out of DNA. Researchers can put the desired gene within a transposons' molecular machinery and expose it to insect cells. Another way is through the use of viral vectors. Similar to transposons, a desired gene can be spliced into a virus construct. Then, viral vectors can be used to integrate into the genome on a random basis.

2. Paratransgenesis

This technique involves altering the flora (or adding completely new flora) in the gut of an insect, but not altering the insect's own genome. The concept was given by Frank Richards in 1996. Researchers can use the interaction between the pathogen and the natural flora of the insect's gut to destroy the disease agent. The kissing bug (*Rhodnius prolixus*) transmits chagas' disease (*Trypanosoma cruzi*). Researchers have been able to alter the bacteria commonly found in the gut of the kissing bug to produce an anti-trypanosome peptide, cecropin. When expressed, this peptide does not harm the insect, but is deadly to the pathogen.

Transposons in *Drosophila* - P element

One family of transposons in *Drosophila melanogaster* is called *P elements*. They seem to have first appeared in the species only in the middle of the twentieth century and have spread through every population of the species within 50 years. A technology to use artificial P elements to insert genes into drosophila by injecting the embryo was thus pioneered by Rubin and Spradling (1982). Exogenous DNA sequences were introduced into the *Drosophila* germ line. A rosy transposon (ry1), constructed by inserting a chromosomal

DNA fragment containing the wild-type rosy gene into a P transposable element, transformed germ line cells in 20 to 50 per cent of the injected rosy mutant embryos. Transformants contained one or two copies of chromosomally integrated, intact *ry1* that were stably inherited in subsequent generations. These transformed flies had red wild-type eye color indicating that the visible genetic defect in the mutant host strain could be fully and permanently corrected by the transferred gene.

An individual *D. melanogaster* may carry TEs from 50 different transposon families with 100 copies per family, representing as much as 20% of the genome (Engels, 1992). The P transposable element has been developed as an important a molecular tool in *Drosophila* genetics. P is a highly mobile class II TE with short inverted terminal repeats that are 31 bp in length. P excision and chromosomal integration is by a cut-and-paste mechanism that creates a duplication of the insertion site. P excision is catalyzed by transposase, the P-element-encoded enzyme required for transposition, at the inverted repeats on the P element. This leaves behind a double-stranded gap that is usually repaired using the P element sequence on the sister chromatid as a template, thus replacing the missing P element (Daniels and Chovnick, 1993).

The excised P element may insert randomly into a new position in the genome, which may cause mutations. P elements were discovered in the 1970s due to their role in hybrid dysgenesis, which occurred when males from a wild population mated with females from a laboratory population. The progeny had numerous genetic defects such as sterility, mutations, broken chromosomes, and developmental abnormalities (Kidwell *et al*, 1997). P transposable elements, present in the wild strain (P cytotype), but absent in the laboratory strain (M cytotype), were found to be the causal agents. When P strain males mate with the M strain females, the P elements are released from regulation of the P cytotype as the sperm enters the egg. Transposase is expressed due to the lack of a repression system and the mobilized P elements cause mutations (Robertson *et al*, 1988).

The first attempts to genetically transform non-drosophilid insects with transposon-based vector systems utilized those that had already become routine for *Drosophila*. These included:

- P system: P-element transposase is a novel site-specific endonuclease in *Drosophila*. P was non-functional outside the Drosophilidae (Handler *et al*, 1993).
- *hobo*: Another transposable element which

became available several years later (Blackman *et al*, 1989). *Hobo* function was limited.

***piggyBac* transposon**

A mobile element, called *piggybac* (discovered by Malcolm Fraser at the University of Notre Dame) is now routinely used for insect transgenesis. The *piggyBac* element was originally identified by its association with a mutation in a baculovirus passed through the *Trichoplusia ni* cell line culture (Fraser *et al*, 1983). This transposon is naturally found in the genomes of some lepidopteran (Cary *et al*, 1989). The *piggyBac* element was first used to transform the medfly, *C. capitata* and more recently it has been used for transformation of many other species (Horn and Wimmer, 2000).

These are class II mobile element which move directly from one position to another within the genome using a transposase to “cut and paste” them within the genome. TTAA-specific, these short repeat elements are a group of transposons that share similarity of structure and properties of movement and were originally defined in the order lepidoptera, but appear to be common among other animals as well. These are extremely useful tools for transformation of animals, particularly insects in the orders diptera, lepidoptera, and coleoptera. Of particular importance to the use of *piggyBac* in insertional mutagenesis studies for functional genomics is the precise nature of its cut-and-paste transposition mechanism. The first definitive demonstration of genetic transformation of an insect by the *piggyBac* transposon was accomplished using the mediterranean fruit fly as a model system (Handler *et al*, 1998).

The *piggyBac* ORF encodes a functional transposase.

Applications of transgenic insects

❖ Industrial biotechnology

Goals

- ✓ Introduce disease resistance genes into silk moths to increase production.
- ✓ Increase number of silk genes in moths to increase yield per moth.
- ✓ Introduce genes from spiders or other silk producing arthropods into silk moths to produce special types of silk.

Silk from spiders

- ✓ 5 times stronger than a thread of steel of the same thickness.
- ✓ 3 times stronger than Kevlar (carbon fibre).
- ✓ Spiders are carnivores and cannot be raised.



Diagram of the 2.47 kb *piggyBac* element showing positions of the inverted terminal repeat (ITR) sequences, sub-terminal inverted repeat (IR) sequences, 1.8 kb open reading frame (ORF) and duplicated TTAA insertion site.

- ✓ Transgenic animals can produce the spider silk protein.
- ✓ Of particular interest is the silk that forms the dragline - unusual combination of strength and extensibility superior to currently known high-performance materials.
- ✓ The gene for spider silk protein was isolated from the Golden orb weaver (*Nephila clavipes*).

A chimeric (hybrid) silk protein has been constructed fusing spidofibroin (spf) proteins comprising one or more repetitive domains from a spider dragline silk protein and amino acid sequences involved in transport and/or processing of expressed silk protein in the insect. The *pbac* system was used to transform *Bombyx mori*. Thus, fibers spun by transgenic moths were a hybrid of SpF (spider) and Fib-H (silk moth). To increase the proportion of SpF in the silk, the amount of endogenous *B. mori* Fib-H was reduced using interference RNA (RNA-i).

GMI involved control of insect pests

1. Transgenic pink bollworm

- Million of male pink boll worm moths were sterilized by irradiation in SIT
- Moths were engineered to contain gene from jelly fish (GFP) and a lethal gene (tTA) is introduced from bacteria (Peloquin, 2000)
- It alters the metabolism of the moth larvae

2. Transgenic mite

- They feed on spider mites, a polyphagous pest.
- First field trial of a transgenic mite- Transgenic predatory mite *Metaseiulus occidentalis*, a predator of spider mites (Presnail, 1996).

3. Transgenic red flour beetle

- It is a worldwide pest of stored products
- Genes responsible for regulating pheromone secretion are mutated
- Specific gene expression is knocked out by RNA interference

GMI for production of economically useful insects

Death of honeybees under rearing conditions is mainly because of entomopathogens and parasites. So genetically

engineered honeybee with gene coding for hDAF are resistant to diseases, parasites and insecticides as reported by Kimura, 2001. Genetic manipulation has resulted in disease resistance and pollination attributes in honeybee (Rothenbuhler, 1979). Silkworm is modified to produce industrial and therapeutic proteins like human growth hormone and human collagen (Kadonookuda, 1995).

Paratransgenesis is another use of transgenic technology in crop protection that attempts to eliminate a pathogen from vector populations through transgenesis of symbiont of the vector. The goal is to control vector-borne diseases. It involves insertion of genes into symbionts that live in host organisms. The first step is to identify proteins that prevent the vector species from transmitting the pathogen. The genes encoding these proteins are then introduced into the symbiont so that they can be expressed in the vector. The final step is to introduce these transgenic symbionts into vector populations in the wild.

The key feature of *symbiotic control*, which uses symbionts to control pests or disease, is identifying a symbiont that has both an intimate relationship (called mutualism) with a diseased host and access to the pest or pathogen attacking it. The difference between symbiotic control and *biological control*, which uses organisms such as parasites or predators to control pests or disease, is the transgenic pest organism acts as a symbiotic control agent itself rather than parasites or predators acting to control the pest. Symbiotic control is different from ordinary microbial pesticides, which are used the same as commercial insecticides, in having greater selectivity and fewer side effects. The symbiotic agent can be designed to affect only the pathogen causing the given disease.

The pink bollworm, *Pectinophora gossypiella*, is a world-wide pest of cultivated cotton. In certain growing regions populations are suppressed by a sterile release strategy. Efforts to improve the sterile insect technique as well as our understanding of lepidopteran biology could benefit greatly from a germ-line transformation system. Peloquin *et al* (2000) reported the transformation of pink bollworm with a *piggyBac* transposable element carrying

the enhanced green fluorescent protein (EGFP) marker gene. This vector-marker system resulted in recovery of transgenics at a rate of approximately 3.5%. Integration of the transforming construct that was typical of *piggyBac* was demonstrated by Southern analysis and sequence determination of transposon flanks. Expression of the EGFP marker was visualized by fluorescent microscopy and Western Blot analysis. Maintenance of transformed strains indicates that the transgene segregates in a Mendelian fashion and has been stable over fourteen generations to date (Peloquin *et al*, 2000).

To achieve the genetic transformation of a mutant *white* of *D. willistoni* using the binary system (vector/auxiliary) introducing a gene marker that codified the protein EGFP through the microinjection of recombinant plasmids in pre-blastoderm embryos. The pattern of expression of the protein in the larva and adult, and the stability of the transformants were analyzed along six generations.

- ✓ *D. willistoni white* mutant strain EM1.00 was used for genetic transformation.
- ✓ The vector pBac [3xP3-EGFP] containing the transposon *piggyBac* inverted terminal sequences (ITRs) necessary for mobility as well as the marker gene EGFP were used.
- ✓ The methodology of microinjection and post-injection treatments were followed.
- ✓ Analysis of transformants: The adult flies outcoming from the microinjected eggs (Go) were backcrossed to *white flies* and the progeny and subsequent generations (G1-G5) were examined to identify the fluorescence expression.

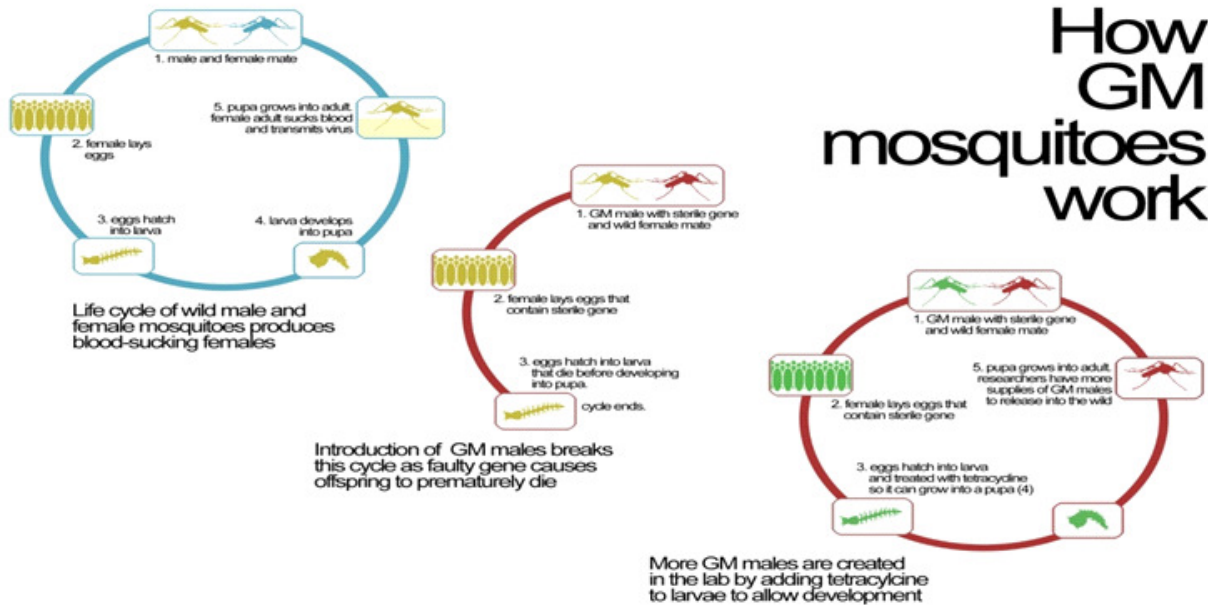
Finokiet *et al* (2007) carried out experiments in which a total of 539 embryos (Go) were injected with mixture of vector and helper plasmids, obtaining 15 larvae and 8 adults. Go adults were backcrossed to *white flies*, six of them were fertile allowing to establish six isolines. Expression of the fluorescent protein EGFP could be observed in the progeny of four of the isolines established. The rate of genetic transformation of the Go adults was of 66.7% which is high when compared with that obtained by other authors for other species. The results of the experiment presented here demonstrated that the transformation system composed by transposon *piggyBac* and marker EGFP was quite efficient to mediate the transformation in *D. willistoni*. Meanwhile, no stable isolines were obtained suggesting a high mobilization of the *piggyBac* in this species. The utilization of a concentration of a plasmid smaller than the one usually employed did not impede the successful genetic transformation of *D. willistoni*, being the species that

showed the highest transformation rate using *piggyBac* as a vector.

Comparisons between transgenic (T) and wild-type *Metaseiulus occidentalis* colonies (COS) were made under laboratory conditions as part of a risk assessment effort prior to proposed field releases. There were no differences between the transgenic T18 colony and the COS strain in the daily egg production, hatchability at three temperatures and four relative humidity (RH) conditions, diapause incidence, or proportion of female progeny produced. *Metaseiulus occidentalis* eggs do not hatch at 38°C under any RH tested, nor at 33.5°C under 100% RH, indicating that high temperature and extreme RH affect egg hatch negatively. At 28.5 and 33.5°C, fewer eggs hatched at 32.5% than at 75.5 and 93% RH. *Metaseiulus occidentalis* cannot survive on any plant tested without prey nor on a diet of pollen alone; adult female mites cannot prey or survive on a diet of eggs and larvae of two lepidopteran species, indicating that the suitability of food sources has not been altered in the transgenic strain. Two subcolonies, derived from two transgenic strains using single females, differed in the rate of egg hatch at 28.5°C under 32.5 and 100% RH, indicating that reduced genetic variation and/or random genetic drift in the two lines may have led to differences in some biological characters (Li and Hoy, 1996).

A transgenic strain of the natural enemy *Metaseiulus* (= *Typhlodromus* or *Galendromus*) *occidentalis* (Acari: Phytoseiidae) was developed using a method termed maternal microinjection. This transgenic strain was released into an experimental site on the campus of the University of Florida in 1996 after extensive reviews by the University of Florida Biosafety Committee, Florida Department of Agriculture and Consumer Services, United States Department of Agriculture Animal and Plant Health Inspection Service, and the United States Fish and Wildlife Service. The short term releases established a precedent for releasing a transgenic arthropod but, at present, no guidelines are available that would allow transgenic arthropods to be released permanently into the environment. Several scientific, environmental, and policy issues must be resolved before transgenic pests or beneficial arthropods can be deployed in practical pest management programs (Hoy, 2000).

Four lines of *Metaseiulus occidentalis* (Nesbitt) were transformed by maternal microinjection. We evaluated the stability of these lines, whether the plasmid DNA was integrated into the nuclear genome, and conducted tests of mode of inheritance to determine whether the transgene was transmitted by males. The putatively transformed lines were identified by standard



Working of GM mosquitoes.

polymerase chain reaction (PCR) in the G_1 generation. After H^{30} generations, the lines were examined by Southern blot hybridization, and the plasmid probe was hybridized to uncut high-molecular-weight DNA from all 4 lines, indicating that the transgene was associated with high-molecular-weight DNA. In restriction-digested Southern blots, 1 of the 4 lines (18) displayed a hybridization pattern consistent with integration of 2 plasmids into the chromosomal DNA. All 4 colonies remained positive by PCR after 150 generations. All 4 colonies examined by RNA-PCR for messenger RNA (mRNA) expression after 100 generations displayed PCR products consistent with transcription of the introduced genetic sequences. In tests of mode of inheritance, plasmid sequences were transmitted by line 18 males, which is consistent with the hypothesis that the plasmid DNA is integrated into the nuclear genome in this line (Presnail *et al*, 1997).

Pest management goals that can be achieved by transgenic methods

Natural enemies used in biological control of insect pests and mites could be modified to enhance their effectiveness in several ways:

- Improve survival of natural enemies in environment.
- Enhance ability to control pests by insertion of stress resistance genes or pesticide resistance genes.
- Modify diapause or temperature tolerance traits.
- Improve effectiveness of natural enemies by altering traits that enhance effectiveness such

as altering sex ratio (more females); alter longevity; fecundity; host/prey specificity.

- Restrict ability to fly (flightness).

Transgenic technology : current state

- ✓ Genetic transformation of non-drosophilid insects is now possible using four transposon-based gene vectors Mariner, Minos, Hermes and *piggy-Bac* transposable elements (Handler, 2001).
- ✓ Used to transform 15 species of insects including Diptera, Hymenoptera, Lepidoptera and Coleoptera.
- ✓ The dominant fluorescent protein marker systems, combined with highly conserved gene regulatory sequences, provide reliable methods for detecting, maintaining and recognizing transgenic insects.
- ✓ One pest, the PBW, has already been registered for testing a fluorescent marking system in a contained release.
- ✓ A malaria vector *Anopheles stephensi*, has been made partially refractory to an infectious pathogen (Ito *et al*, 2002).

The potential benefits of GM insect strategies

Proponents of GM insects consider them to be a tool to complement existing control methods. Several unique benefits of GM insects have been proposed:

- ✓ They would target only a single insect pest species, leaving beneficial insects unharmed
- ✓ By using insects natural propensity to find one

another, pest populations inaccessible to traditional control methods could be eliminated

- ✓ GM insects could reduce the need for insecticides and any associated toxic residues in the environment
- ✓ When used in disease control programmes GM insects would protect everyone in the release area, irrespective of socio-economic status.
- ✓ Disease control using GM insects would require less community involvement and so would be less vulnerable to the failure of individuals to participate in a control programme.

Advantages of GM insects

1. In Public Health

Malaria factsheet – Statistics

According to the World Health Organisation, there are up to 500 million clinical cases of malaria each year resulting in approximately 2.7 million deaths. This disease is responsible for over a million deaths every year mainly among young children in Africa. It is one of the top priorities of the World Health Organization. The malaria situation around the world is worsening. *Plasmodium falciparum* has become resistant to the most common antimalarial drugs in most of its area of distribution. Mosquitoes are also becoming more resistant to chemical insecticides. According to material from Third World Network Features, in Africa alone, direct and indirect costs of malaria amounted to USD 800 million in 1987 and were expected to reach USD 1.8 billion annually by 1995.

The prospect of eliminating malaria moved a step closer with the announcement of European scientists creating the world's first transgenic malaria mosquito. Scientists at Imperial College, London, and the European Molecular Biology Laboratory, Heidelberg, report in *Nature* how they have successfully inserted a foreign marker gene into the mosquito genome. This achievement means that transmission of the disease could be controlled through manipulation of mosquito genes; to disrupt the insect's interaction with the malaria-causing parasite, by altering its choice of blood target from man to animal, or by selectively creating sterile male mosquitoes. The researchers borrowed gene transfer technology successful in another species, the fruit fly *Drosophila*, and adapted it to work in the mosquito species *Anopheles stephensi*.

The transgenic mosquito the team developed was made to carry an extra gene that produces a green fluorescent protein. This protein is used because it acts

as a highly visible marker (under ultraviolet light) to indicate which mosquitoes have successfully incorporated the foreign genes.

- ✓ The transgenic mosquitoes have high survival rate and lay more eggs.
- ✓ Those mosquitoes have glowing eyes under UV light due to the presence of green fluorescent protein (GFP) inserted into it.
- ✓ *Anopheles stephensi* is one transgenic mosquito species to resist malaria (Catteruccia *et al*, 2003).

2. Agriculture

Pink boll worm

- Sterile insect technique (SIT) program
- Protects more than 900,000 acres of cotton.
- Million of male pink boll worm moths were sterilized by irradiation (Peloquin, 2000).
- Moths are engineered to contain gene from jelly fish (GFP).

Mediterranean fruit fly

- Males are sterilized by irradiation prior to release so that female reproduction hinders.

3. Other associated benefits

Recent developments in scientific techniques have allowed researchers to create GM silk worms, harnessing the strong protein production of the worm for production of other proteins. One group recently created a gm strain of silkworms that produce human antibodies (Park *et al*, 2009).

Possible Risks of GM Insects

The use of GM technologies is controversial. Some organisations, such as GeneWatch UK and EcoNexus, that monitor the use of genetic technologies, fear that reliance on high-tech solutions, such as genetic modification, detracts from more effective but poorly deployed measures to combat the harm caused by insects. Furthermore, environmental NGOs such as Greenpeace suggest that GM insects could have unintended and wide ranging impacts on the environment and human health due to the complexity of ecosystems and the high number of unknown factors, making risk assessment difficult. They have raised several concerns about the release of GM insects:

- ✓ New insects or diseases may fill the ecological niche left by the insects suppressed or replaced, possibly resulting in new public health or agricultural problems

- ✓ The new genes engineered into the insects may jump into other species, a process called horizontal transfer, causing unintended consequences to the ecosystem.
- ✓ Releases would be impossible to monitor and irreversible, as would any damage done to the environment.

1. Instability of the introduced genes

- Transgenes were reported to get rapidly lost under field conditions (Hoy, 2000).
- Experimental release of transgenic predatory mites showed that very few individuals contained the transgene only after three generations while in laboratory strains, it was persistent over one fifty generations.

2. Horizontal gene transfer

- It could occur from one insect population to another of the same species, or from one insect species to another, or to other organisms in the environment.
- Difficult to quantify this risk
- The whole topic of HGT in insects has received limited scientific attention until relatively recently.

3. Gene silencing

- Multiple mechanisms of transgene silencing occur in *D. melanogaster* (Dorer and Henikoff, 1994).
- It could reduce the effectiveness of transgenic insects after their release in pest management programs.

4. Disruption of ecosystem services

- The potential effects of transgenic insects on ecosystems is a big topic and difficult to evaluate using laboratory tests (Hoy, 2000).

5. Poor fitness

- It is due to the result of introducing transgene thereby making the recipient weaker (Atkinson, 2001).
- Laboratory rearing is not compatible with competitive behavior of insect.

6. Environmental risks

- Once they released, they can't be taken back so lots of investigation and regulation should be done for this research.

Commercially released GM insects

- Predatory mites- in 1997 in USA

- Pink bollworm- in 2001 in Mexico
- Anopheles mosquito- in 2002 in New Delhi, India
- Screw worm fly- Kenya and Central America

The regulation of GM insects

Existing Regulation

At the international level, the Cartagena Protocol on Biosafety applies to the transboundary movement, transit, handling and use of all GM organisms (GMOs) that may "have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health". The ability of insects to travel long distances and to cross international borders means regulation of transboundary movement will be required, particularly in the case of a self-propagating population-replacement strategy that could spread over entire continents.

The release of a GM insect within any EU member state is controlled by European Directive 2001/18/EC, known as the Deliberate Release Directive, which regulates deliberate release of all GMOs into the environment. Legislation regulating GMOs has been widely initiated in the rest of the world since the ratification of the Cartagena programme, but is often poorly implemented. In Africa, the African Union has drafted the African Model Law on Biosafety, and recently individual countries, such as Kenya with its Biosafety Act of 2009, have created legislation regulating the release of GMOs into the environment. However, in some nations GMO regulation remains undeveloped (Oliver, 2010).

CONCLUSION

The success of Integrated Pest Management (IPM) programs is strengthened by the compatibility of chemical and biological control methods used in the system. This is challenging for complex agricultural systems where it is not possible to control key pests by methods other than chemical control (Hoy, 2000), which can result in incompatibility of biological and chemical control strategies causing target-pest resurgence, secondary pest outbreaks, and pesticide-resistant pest arthropods. The use of genetically transformed insects may provide new strategies for insect pest management (Handler, 2001).

Experimental releases of genetically modified (GM) insects are reportedly being evaluated in various countries, including Brazil, the Cayman Islands (United Kingdom), France, Guatemala, India, Malaysia, Mexico, Panama, Philippines, Singapore, Thailand, the United States of America, and Vietnam. GM mosquitoes (*Aedes aegypti*) have already been released for field trials into inhabited

areas in the Cayman Islands (2009), Malaysia (2010–2011), and Brazil (2011–2012) (Revees *et al*, 2012).

Public opinion of GM technologies varies greatly between nations. In the EU, public perception of GM technologies largely has been defined by the GM crop debate. The lack of public acceptance of GM technologies led to a 12 year *de facto* moratorium on approval of any GM crops in the EU. This ended only in March 2010, though “delays remain” and makes release of a GM insect in the EU in the near future unlikely. Public consultations on attitudes to GM insects have yet to be conducted in many countries. In the EU, polls have shown a year on year increase in positive responses to GM technology, especially its medical applications¹¹. In other nations, particularly those most likely to benefit, the public response to GM technologies has often not been investigated. Many communities are sceptical about the benefits and regulation of genetic modification which is often perceived as ‘unnatural’ and as such undesirable. Engagement with the communities that will be affected by GM insects will be needed if the technology is to be accepted by the public and eventually deployed. This process is already ongoing in countries where releases of GM mosquitoes for disease control are planned¹². For example, the communities around a test-site in Mexico have been involved with the international collaboration responsible for the last four years. Engagement with communities and local scientists in Asian and African countries is being led by bodies such as the WHO’s Special Programme for Research and Training in Tropical Diseases and those that fund research into GM mosquitoes, including the Grand Challenges in Global Health and the Wellcome Trust. It is hoped that this early engagement, combined with the potential benefits of GM insect technologies, will lead to their social acceptance.

Insects are essential to global ecology and show remarkably varied adaptations to their environment. They are also responsible for economic and social harm worldwide through the transmission of disease to humans, animals and damage to crops. Their genetic modification has been proposed as a new way of controlling insect pests. In the midst of millions of people suffering from hunger and diseases, expanding this novel approach of GM insects can be the panacea with appropriate social policies. However, regulatory guidelines governing the use of such technology have not yet been fully developed. In my view, the advantages of GM insects outnumbers the disadvantages of this technology but sound research on the public safety and sustainable ecological balance is necessary so that technology might not overestimate the right of future generation in the matter of preservation of

our ecology and self-sustained nature.

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