**Genetic Engineering of Insects and Their Application**

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**INTRODUCTION**

Genetic engineering or genetic modification is the applied biotechnology where the entire or some part of the DNA makeup of an organism has been changed in the laboratory using different techniques. The foremost recombinant DNA molecule was synthesized by [Paul Berg](https://en.wikipedia.org/wiki/Paul_Berg) in 1972, where he combined the DNA molecule from the monkey virus [SV40](https://en.wikipedia.org/wiki/SV40) with the [lambda virus](https://en.wikipedia.org/wiki/Lambda_phage). Era of biotechnology was begun in 1973 with the creation of functional bacterial plasmid as the experimental result of Cohen and Boyer under in-vitro condition (Cohen *et al*., 1973). In 1980s for biotechnological uses first GM insects was created (Marx, 1987). Evolution of applied biotechnology have occurred during last thirty years, in 2009 first approval of an anti-clotting factor produced in goat milk, was given by Food and Drug Administration which was first approved pharmaceutical produced by a GM animal (Pollack, 2009). Considering the molecular complexity the GM insects occurred between the pharmaceuticals providing GM animals and GM crops. Before applying of rDNA technology several scientists applied sterile insect technique for managing worst screwworm fly in southern US during 1950-60s (Bushland *et al.*, 1955). Genetically modified insects are those insects which have been genetically modified by mutagenesis or more precisely can be said that, through transgenesis or cis-genesis for various purposes like agricultural production, oil production, tackling health issues and pest management through biotechnology. Genetically modified insects are made transgenic by inserting one or more DNA sequences from other organisms into their genome. The reasons behind the production of GM insects are managing the agriculturally important insect pests, maintaining quality of produce, managing environmental and human health issues and reducing the harmful impact of synthetic pesticides used in pest management systems. GM insects are developed for achieving the goal for managing the insect vectors of human diseases like malaria and dengue fever. It is well proven that modification of insects and other arthropods in the genetic level can be effective strategy for studying of development and evolution at molecular level (Horn and Wimmer, 2000). These genetic modification strategies can be considered as the effective tool for solving several agricultural and health issues which is caused by certain insect. The insects can be altered in the genetic level with suitable desired traits and released in the nature cautiously (Irvin *et al*, 2004). The utilization of genetically modified (GM) insects offers a novel and innovative approach to address the insect-vector of human diseases and losses due to insect pest in crops. Though the risk assessment and acceptance of the risks associated with the GM insects compared to the potential gain is the main determining factor in the way of developing GM insects.

**History of Genetic Engineering in insect**

Prior to the 1950s, New World Screw-worm *Cochliomyia homnivorax*, became the major health hazarding factor of cattle which was extremely difficult to control. Raymond Bushland and Edward Knipling hypothesized that if the male flies can be sterilized, then a single male would prevent several females from laying viable eggs and eventually the population would shrink. Besides this the sterile males would outnumber the fertile males also. Experiment for these purposes gamma irradiation, UV rays and mutagens like ethyl methyl sulphonate were used with an aim of generating the insect population having high sterile to fertile ratio which was achieved by using gamma irradiation, UV rays and mutagens like ethyl methyl sulphonate. The concept of genetic control of insect pest was given by E. F. Knipling which marks the history of GM insects (Joe, 2010). Genetic transformation of insects by insertion of DNA particle from external sources was first performed in a mutant line of a stored grain pest *Ephestia khuniella* which had no scale in their body. In 1965 when external wild type DNA was introduced in the pest then the transformed adults produced wing scales (Atkinson and James, 2002). Previously genetic change through germ-line transformation was carried out in *Drosophila melanogaster* using transposable *P* element (Rubin and Spradling, 1982). Gene encoding the wild colour was *rosy*+ which was successfully introduced in the *rosy* mutant *Drosophila* using a vector namely transposable *P* element. In this way wild red eye color got stably introduced in the mutant strain and successfully inherited, though this transformation was restricted in few species of subgenus *Sophophora* under the genus *Drosophila* (Handler *et al*, 1993). After 13 years later first non-drosophilid insect, that was Mediterranean fruit fly, *Ceratitis capitata*,was transformed using a transposable element (*minos*) (Loukeris *et al*, 1995).

**Need for genetic eengineeing in insect**

* Area-wide management of pests of halth and agricultural importance
* Can be used as bioreactors to produce pharma products
* Developing virus-resistant lines in economically important insects
* Enhancement of quality agricultural production, productivity
* Beneficial management of public health
* Improving disease resistance ability, pollination attributes in honey bees and improving silk worm moth for better quality silk production. (Gopinathan, 1992).

**Strategies of genetic engineering in insects**

Production and application of GM insects in different field is based on several strategies which can be classified in the following way

**Self Limiting:**

The principal goal in the self limiting strategy is population suppression. In this strategy, expectation suggested that the novel characteristics should face more or less rapid disappearance after releasing in the field. So the modified desired trait can be maintained only after releasing the modified additional insects periodically. One of the popular examples of self limiting strategy is the sterile insect technique (SIT) where genetically sterile male is released in the field which is regarded as low risk and least controversial method of genetic control (Camilla *et al.*, 2012). Self-limiting methods also focused on other methods like female killing methods, sex-ratio alteration and delayed in conditional lethality. (Foster *et al*., 1988; Fryxell & Miller, 1995; Schliekelman & Gould, 2000a; Schliekelman & Gould, 2000b; Schliekelman *et al*., 2005; Bax & Thresher, 2009).

**Self sustaining**

Self-sustaining strategies are designed to focus on the modified traits to be indefinitely persisting in the environment and spread in the native population and increase its frequency and geographical area over time. This strategy mainly focused on control of insect vectors of diseases of human being (Marshall & Taylor, 2009). Such self-sustaining strategies aimed to replace the population of harmful insects into a non-harmful or less harmful form, as for example it may be a form that has less ability for transmission of one or more pathogens. The goal can be achieved by transgenesis where a transgene may prevent *Plasmodium* species to infect the vector mosquito. For effective outcome, huge proportion of this desired gene would have to be present and persist in the population of vector mosquitoes in a particular area and the transformed species should established in the wild population after release.

**Physical Methods of genetic transfer**

**Microinjection:** Microinjection was first described by Lin in mouse egg in 1960. It is a physical process of delivering external or foreign DNA inside the cell or egg or oocyte or embryo of an animal. In this method a glass micropipette is used to inject a liquid substance containing foreign DNA under the stereomicroscope. Here a Holding pipette is used to hold the particular target cell aimed to insert the foreign DNA of desired trait, the cell is held at its tip when the cell gently sucked. Then the tip of the micropipette is injected through the membrane of cell. In this way the material of the needle is delivered into the cell cytoplasm and after that it is taken out. The inserted DNA tried to integrate itself with the nuclear DNA of target cell randomly and the expression of this foreign DNA is only possible when it successfully attached with the proper and suitable promoter sequence. Sometimes the contents are also delivered in the intercellular space also. So it is a well adapted delivery system routinely used for transformation of animal cells, tissues, eggs and embryos by inserting the desired genetic molecule as like DNA, RNA, proteins and macromolecules in a direct way.

**Biolistics :** It is also known as particle (heavy metal) mediated gene transfer.Sometimes it is called gene gun or a biolistic gene delivery system, which is mainly devised for delivering foreign DNA into plant cells. In this system the instrument composed of a bombardment chamber, connective tubing for attaching with the vacuum source and other parts like helium regulator, solenoid valve etc. to for attaching and delivery of the helium kept under high pressure, in the main unit. The desired gene (DNA particle) is coated on the particle of heavy metal like gold or tungsten. The particles coated with external DNA are propelled in the target cell at higher velocity and release the DNA particle which integrates itself with the genome of the particular cell.

**Lipofection :** It is known as liposome transfection, in this method liposomes are used for transforming a cell by inserting foreign genetic material. Liposomes are small vescicle and capable of merging themselves with the cell membrane as both of them have phospholipid bilayer. Lipofection generally uses positively charged (cationic) liposomes to form a transfection complex with the negatively charged (anionic) genetic material with desired traits. This complex then fuses with negatively charged cell membrane and get entry inside the cell through endocytosis. Then it go through the endosomal pathway and releases the foreign genetic material and then the external DNA enter inside the cell genome.

**Electroporation :** It refers to the transformation of cell via direct gene transfer action. In this method target cells along with desired DNA are exposed to very high voltage electrical pulses (4000 – 8000 V/cm) for a very brief time span (few milliseconds). As a result formation of transient pores in the plasma membrane takes place which can be called as electropermeabilization and by this pores DNA enters inside the target cell and get integrated with cell nucleus. Success of this process depends on salt concentration. Higher salt concentration may cause electrical discharges. Cells arrested at metaphase stage are suitable for electroporation as nuclear envelope is absent in this stage as well as unusual permeability of plasma membrane.

**Different Methods Used in Genetic Modification of Insects**

**Transposable elements:** Transposons or transposable elements are a kind of selfish genes and they are pieces of DNA mobile in nature and do not stay in fixed location of a genome (Kidwell & Lisch, 1997; Liao, 2000). The movement of rare sequences of DNA from one position to another positon within the genome of a single cell is often termed as transposition. Through this action they can they can derive mutations in the genome. Transposons are also termed as “jumping genes”, as they are mobile in nature. First discovery of these jumping genes was done by Barbara McClintock for which she got Nobel Prize in 1983. There are a variety of mobile genetic elements, and they can be grouped based on their mechanism of transposition.

**Mechanism-**

Integration of a transgene by the transposition needed a donor and a helper plasmid among them no one is autonomous. Desired gene to be integrated and a detectable marker for visual identification are held by the donor plasmid within its functional terminal inverted repeats (TIR). Being absence or presence of defective TIRs the helper primer cannot start transposition; only function of them is encoding the transpoae enzyme required for transposition. It was also found that using of capped mRNA replacing the helper plasmid provided more effective results in some cases (Pavlopoulos *et al.,* 2004). Several studies showed that microinjection in early embryos provided better result where plasmids are provided in to the germplasm of syncytial blastoderm stage as because the primordial germ cells are formed there and nuclei of some cells taken up the plasmids with gene of interest. After developing as an adult, the concerned transpoase enzyme recognizes the particular TIRs and start transposing the gene of interest that is constructed from donor plasmid on the particular chromosome. In this way the inserted transgene is carried by the genome of some of the germ-line cells. When the offspring get the inserted transgene through the cells then they stably become transformed. Due to low efficiency of genomic transgene insertion, suitable marker is needed for proper and easy identification of the transformed individuals.

**Method of Transformation**

Insects normally have four lifestages viz. egg, larva, pupa and adult, though there are so many variation as the entire insect does not possess all these four stages in their life; some of them may be absent. For successful transformation generally eggs are used, though adults are also in use less frequently. Most common method for delivering gene of interest within the egg of the target species is microinjection system which comprises of a stereozoom microscope, a mechanical stage, micromanipulator and a mechanism for DNA injection (manual or electronic air-pulse system).In this process the needle is aligned along with micromanipulator. Micropyle end of the egg is oriented towards the needle by moving the mechanical stand. DNA carrying the desired gene for transformation flanked by TIR along with transpoase enzyme is transferred to the region containing the germplasm of the early embryo. During the embryonic development transpoase enzyme acts on the TIR and initiate transposing of the gene of interest on a chromosome. As a result the offspring born from that egg is become genetically modified. There are two ways of modification through microinjection; in one method the trans-gene is injected into the follicle of ovary prior to oviposition and in another method the foreign genetic material is injected in the haemocoel of female insect for uptake of the genetic material into egg follicles along with vitelline.

**Classification of Transposable elements**

There are three classes of transposable elements (Pimpinelli *et al*., 1995)-

* Class I elements – These element mediate transposition through reverse transcription (They become as like RNA for transposition)
* Class II elements- These element mediate transposition transposition directly between DNA, travelling from one position to another position completely (they does not copy) (Pimpinelli *et al*., 1995). In insects for germ-line transformation generall Class II elements are used
* Class III elements- They are generally termed as miniature inverted-repeat transposable elements (MITES); they are small elements incapable of encoding any protein but they are able to non-replicative relocation to a new insertion locations.

**Different Transposable Elements:**

***Hermes***

This element helongs to the hAT family closely related to hobo, first discovered in the housefly, *Musca domestica* (Atkinson *et al*., 1993; Warren *et al*., 1994). Hermes and piggyBac, are mainly used for transformation of insects other than nondrosophilids, though they are extensively used and testing results indicated their applied purposes. These elements had been used for transforming several insects like Yellow fever mosquito, *Aedes aegypti* (Jasinskiene *et al*.,1998) ,Southern house mosquito, Culex quinquefasciatus (Allen *et al*., 2001), Mediterranean fruit fly, Ceratitis capitata (Michel *et al*., 2001), Stable fly, Stomoxys calcitrans (O'Brochta *et al*.,2000), Squinting bush brown butterfly, *Bicyclus anynana* (Marcus *et al*., 2004) Red flour beetle,*Tribolium castaneum* (Berghammer *et al*.,1999) etc.

***Mariner and Minos***

The mariner element was first recognized from Drosophila mauritiana (Jacobson et al., 1986). Among the transposons used as vectors in non-drosophilids, *mariner* was the first to be discovered, they belongs to Tc family. This element is 1,290 bp long and comprises a 21-bp imperfect terminal repeat. Insects transformed using this element are Yellow fever mosquito, *Aedes aegypti* (Coates *et al*., 1998), Housefly, *Musca domestica* (Yoshiyama *et al*., 2000) etc.

 Minos was 1st successfully used for germline transformation of non-Drosophilu insects (Loukeris *et al*., 1995). It has 1.4-kb length and a characteristic long inverted terminal repeats of 100 bp with 60-bp intron. It belongs to Tc1/mariner super-family (Franz *et al*., 1994). This element was used for germline transformation of Indo-Pakistan malaria mosquito, *Anopheles stephensi* (Catteruccia *et al*., 2000), Silkworm, *Bombyx mori* (Uchino *et al*., 2007).

***PiggyBac***

Most commonly used element for insect transformation. piggyBac inserts exclusively into TTAA target sites (Cary *et al*., 1989). The size of this element is 2.5 kb along with 2.1-kb open reading frame designed for encoding a transpoase enzyme and 13-bp inverted terminal repeat. Insects has been transformed using this element are Pink bollworm, *Pectinophora gossypiella* (Peloquin *et al.*, 2000), Silkworm, *Bombyx mori* (Tamura *et al.,* 2000), Harlequin ladybird, *Harmonia* *axyridis* (Kuwayama *et al*., 2006), Sawfly, *Athalia rosae* (Sumitani *et al*., 2003), Housefly, *Musca domestica* (Hediger *et al*., 2001), New World screwworm, *Cochliomyia hominivorax* (Allen *et al*., 2004), Mediterranean fruit fly, *Ceratitis capitata* (Handler *et al.,* 1998), Queensland fruit fly, *Bactrocera tryoni* (Raphael *et al*., 2010), Oriental fruit fly, *Bactrocera dorsalis* (Handler & McCombs, 2000), Mexican fruit fly, *Anastrepha ludens* (Condon *et al.*, 2007), Asian tiger mosquito, *Aedes albopictus* (Labbé *et al*., 2010), Indo-Pakistan malaria mosquito, *Anopheles stephensi* (Nolan *et al*., 2002), New World malaria mosquito, *Anopheles albimanus* (Perera *et al*., 2002), African malaria mosquito, *Anopheles gambiae* (Grossman *et al.*,2001) etc.

**Paratransgenesis:**

The principal idea behind paratransgenesis is the reduction of disease spreading ability and competence of a vector by modifying the endosymbiont at genetic level. There is a range of possibilities for effective results which depends on how much tightly the endosymbiont is associated with its host insect. *Wolbachia* species is the popular bacteria that occupy the intracellular space of the insect cells and they are not free living. They are non-infectious in nature transmitted from one individual to another individual by vertical transmissions only that means from mother to offspring. *Wolbachia* is used in a non-GM strategy like in Incompatible Insect Technique (IIT) which can be said as a variant of SIT as described by Chambers *et al*. (2011). In IIT the principal mechanism is based on cytoplasmic incompatibility, induced due to the action of an intracellular bacterium *Wolbachia pipientis* which results in embryonic mortality (Laven, 1967; Brelsfoard *et al*., 2008; Alphey *et al*., 2010). Induction of refractoriness to dengue virus was done using *Wolbachia* under open field condition in Australia (Hoffman *et al*., 2011).

*Wolbachia* can be found in various insects. Vector of dengue virus *Aedes aegypti* does not carry *Wolbachia* and does not normally get infected with the bacteria. *Wolbachia* are transmitted maternally, like mitochondria, but manipulate, the host’s reproductive biology in such a way that they tend to spread through the species. In the presence of *Wolbachia* dengue virus cannot multiply inside the mosquito. *Wolbachia* also induces feminization that means transformation of genotypic male into phenotypic female. They can also modify within male sperm and also reduces egg number in host. *Wolbachia* infection causes several effects like altering the biting position, affects nutritional status of the host, reduces the lifespan of host, affect brain tissues of the host, prevent virus multiplication and indirectly kill the virus.

**Release of Insects Carrying a Dominant Lethal (RIDL)**

Sterile insect technique (SIT) for pest management employs liberation of sterile irradiated males into the native habitat. In this technique female elimination is required as they do not perform any function in control mechanism. For fulfilling this requirement various methods like mechanical sex separation methods using pupal mass, time of adult emergence etc are employed with unsatisfactory outcomes. Due these processes induced chromosomal aberrations may occur which reduces overall fitness of the transformed insect. In this context an alternative way was demonstrated by Thomas *et al*. (2000) which was referred as ‘release of insects carrying a dominant lethal’ (RIDL) in *Drosophila melanogaster*. In this method a transcriptional control element was used to derive the expression of the antibiotic, tetracycline repressible trans-activator fusion protein, tTa. When antibiotic tetracycline is absent, tTa will mediate the expression of any gene that is under control of tetracycline repressive element, tRe which is designed elimination of females.

The tTA is the main driving factor the system, which is a fusion protein that is, responsible for combining the sequence-specific tetracycline-repressible DNA binding properties of the Tn10 TetR protein with the eukaryotic transcriptional activation properties of Herpes virus VP16. In the absence of tetracycline, this protein will bind to the tetO sequence and activate transcription from a nearby minimal promoter. Here a cell lethal effector gene is also placed under the control of tetO. There are several number effector genes are available and they should kill the cell in case of over expression.

Female-specific promoter is required for making the system female-specific, where tTA protein is placed under the control that promoter. As a consequence tTA protein expression occurred only in females. As a result males can survive with or without tetracycline, whereas females need tetracycline for survival and in absence of tetracycline they got killed. In an alternative way Thomas *et al*., 2000, used an effector gene that only kills females. They used a sex determination gene, *msl-2*, whose expression is only restricted to male. Ectopic expression in females resulted in over-expression of X-linked genes and that was lethal.

Several of these RIDL/fsRIDL strains have passed through various assessment processes for checking their potentiality for applying in the field. Characteristics that were considered for further assessment in laboratory for checking the future performance are as longevity, male mating competitiveness and penetrance of the engineered trait when reared on natural host plants (compared to artificial diets). Protocols for these experiments have typically been developed and validated for the SIT, particularly with Tephritid fruit flies. Survival on different crops and trials on mating competitiveness can be conducted in field cages. Then greenhouse trial can be conducted for checking whether the RIDL insects will suppress the wild population. In addition to population suppression, RIDL also provides effective tool for insecticide resistance management. There is, therefore, scope for synergistic use of fsRIDL and insecticides, with potential to reduce overall insecticide use and protect efficacy of valuable and effective modes of action.

This genetic technology proved greatest potential for controlling several vector mosquito like *Aedes aegypti* and other agriculturally important pest like Medfly, olive fly, pink bollworm, diamondback moth and *Ae. Albopictus*. mFurthermore, cross-species function of RIDL and fsRIDL systems – in Tephritid fruit flies, mosquitoes and Lepidoptera - demonstrates that this technology should be relatively easily transferred to other target species in the future, offering a new pest control tool for wider implementation of IPM in agriculture and public health.

 

 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.



# Principle of the release of insects carrying a dominant lethal gene (RIDL). (A) scheme of the transgene. The tetracycline activator variant (tTAV) protein binds to its own promoter, activates its own transcription and perturbs overall gene expression in the cells, resulting in mosquito death, unless tetracycline that binds and inactivates tTAV is provided. (B) During mass rearing in the production unit, mosquitoes develop normally in the presence of tetracycline. For an intervention, males are sorted at the pupal stage (based on the smaller size of male pupae). Once released, they mate with wild females whose progeny will die due to unrestricted tTAV activity.

 Source: *Jérémy Bouyer and Eric Marois,*

**Sperm-mediated transformation:**

The standard procedure for introduction of foreign plasmid DNA into the genome of an organism requires microinjection of that DNA in the early stage pronucleus (mice; Palmiter & Brinster, 1986), into developmental stage containing one-two cell only (zebrafish; Fadool *et al.*, 1998), or inside the egg at early embryonic stage (fruit flies; Rubin & Spradling, 1982). Previously importance was given for transforming honeybee eggs by plasmid DNA through microinjection or lipofection (Bachiller *et al.,* 1991). Though this gave satisfactory result but rearing of transformed honey bee embryos into adults was very much troublesome as the rejection rate of these manipulated embryos by worker bees was very high. The worker honeybees can detect the abnormalities; as a result of this the nursing bees destroy the manipulated embryos. Therefore several researches suggested for using of sperm for delivery of plasmid DNA (Zani *et al.,* 1995; Shamila & Mathavav, 1998). This method comprises of artificial collection of sperm from several drones, followed by inoculation of plasmid DNA and then transfer of the sperm into the oviducts of a virgin honey bee queen. The sperm then migrate into the spermatheca where they are stored for months to years. The use of sperm-mediation for introduction of plasmid DNA would take advantage of the in vivo colony rearing conditions for transgenic progeny, as well as not normally being physically damaging to honey bee queens. It was observed that DNA can bind in the exterior of the sperm, hence can be carried into the egg (Atkinson *et al.*, 1991). Successful transformation of *Bombyx mori* has been achieved by injecting the foreign DNA into the testis of larvae (Shamila & Mathavav, 1998).

**Application of GM insect**

* **Public health issues**
* **Genetically modified malaria causing mosquito**

Mosquitoes are genetically modified to produce protein which causes disruption of life cycle malarial by introduction of Gene SM 1. This gene also prevents entry of malarial parasit inside the mid gut and reaching salivary glands. Green fluorescent protein (GFP) inserted into transgenic mosquitoes for their identification which makes their eyes glow green under UV light. 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. Defensin gene obtained from *Aedes aegypti*, was placed with the help of vitellogenin promoter from this species, that was introduced again into *Aedes aegypti* by transposition using *Hermes* 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. 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 *Anopheles stephensi*. Attacking Plasmodium in the midgut, or at the midgut/ hemocoel boundary, has been a favored site of transgeneexpression 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.

* **Chagas disease**

Pathogenic endosymbiont, *Rhodococcus rhodnii* is found in the hindgut of the triatomine bug, *Rhodnius prolixus*, which is the vector of medically important pathogen *Trypanosoma cruzi*. The normal function of *R. rhodnii* is to make available vitamin B complex which is otherwise unavailable to the host. *R. rhodnii* is acquired by the nymphs of *R. prolixus* through a unique feeding behaviour called coprophagy. *R. prolixus* is made refractive to *T. cruzi* by genetically modifying *R. rhodnii* to express an antitrypanosomal peptide or a transmission-blocking antibody. Rhodnius rhodnii is essential for the survival of R. prolixus, which acquires its symbionts as a nymph through consumption of triatome feces. In a proof-of-principle study, a transgene encoding an antibody fragment was inserted into R. rhodnii, which in turn was stably integrated into the host [microbiota](https://www.sciencedirect.com/topics/immunology-and-microbiology/microflora) by placing the transformed bacteria into fecal matter, which were then fed by R. prolixus nymphs. The transformed R. rhodnii successfully secreted the antibody fragment into the midgut of R. prolixus demonstrating this approach is feasible.

### Diamondback moth-

### The moths were engineered by assembling a “lethality gene” called tetracycline transcriptional activator variant (tTAV), by combining DNA from the bacterium Escherichia coli and the herpes simplex virus; then they added it to the insects.The idea is that when modified males mate with females in the wild, they pass on their tTAV gene. The gene prevents the female offspring from developing, and they die as larvae. But male offspring survive and half inherit tTAV. After these males grow up and mate with other wild insects, the next generation of female offspring also dies, further shrinking the population. The Oxitec insects carry a gene for a fluorescent marker as well, allowing them to be identified in the wild. The modified males have another attractive trait. They could help maintain the effectiveness of insecticides and genetically modified crops the diamondback moth has evolved resistance to. That’s because the modified males added to a field don’t have the resistance genes, as they were bred in the lab from a susceptible strain.

* **Mediterranean fruit fly**

The Mediterranean fruit fly is a global agricultural pest. They infest a wide range of crops (over 300) including wild fruit, vegetables and nuts, and in the process, cause substantial damage. GM-males have been derived by transgenesis which have a lethal gene that prevents female development and kills them in a process called "pre-pupal female lethality". After several generations, the fly population diminishes as the males can no longer find mates. To breed the flies in the laboratory, the lethal gene can be "silenced" using the antibiotic [tetracycline](https://en.wikipedia.org/wiki/Tetracycline).

 Dead fly larvae could be left inside crops. Helen Wallace from [Genewatch](https://en.wikipedia.org/wiki/Genewatch), an organisation that monitors the use of genetic technology, stated "Fruit grown using Oxitec's GM flies will be contaminated with GM maggots which are genetically programmed to die inside the fruit they are supposed to be protecting". She added that the mechanism of lethality was likely to fail in the longer term as the GM flies evolve resistance or breed in sites contaminated with tetracycline which is widely used in agriculture.

* **Pink boll worm**

The reserach teamof Thomas Miller at theUniversity of California, Riverside, USA worked on management of the pink bollworm, *Pectinophora gossypiella* on cotton, by releasing genetically engineered *P. gossypiella* populations that has *Notch* mutant gene. The normal *Notch* gene is responsible for egg development at warm temperatures but prevents the same at cool temperatures. Thus the progenies of mating among mutant and wild population will have less fecundity and thus fail to perpetuate in due course of time. This study has reached a stage of confined field trials.

**Genetically modified Trichogramma**

|  |  |  |
| --- | --- | --- |
| Gene | Source | Against |
| Parathion hyrdolase gene  | *Pseudomonas diminuta & Flavobacterium*  | Organophosphate   |
| Acetylcholine estrase gene  | *Drosophila melanogaster & Anopheles strephansi*  | Organophosphate  |
| Esterase B1 gene  | Culex sp | Organophosphate  |

**Application in Sericulture**

Genetically modified silkworm was modified for the production of industrial and therapeutic proteins like human growth hormone and human collagen. Silk glands of modified silkworm generally express the introduced L-chain gene and GFP. Cocoons produced from modified insects contain recombinant human collagen Spider milk (protein) produced from modified silkworm larvae are used to make bullet proof vests, parachutes and artificial ligaments. (Lewis,2006).

EGT gene prevents the transgenic silkworm from molting and interruots metamorphosis from pupae to adult. (Zhang, 2012). The plasmid DNA was introduced into silkworm eggs by sperm-mediated gene transfer.

**RELEASED COMMERCIALLY**

* Predatory mites – In 1997 in US
* Pink bollworm – In 2001 in Mexico
* Anopheles mosquito – In 2002 in New Delhi and UP
* Screw worm fly – Exported from Libya to Kenya and Central America

**Conclusion**

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. There is great scope in future for popularizing and applying the concept and principles of respective GM technology for modifying the insects of agricultural and medical importance in our country that will boost the economy as well the health conditions of the citizen.

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