# Genetic Engineering of Insects

*R Asokan*

**Insects, which constitute one of the most abundant groups of living creatures on Earth, are significant to human life in numerous ways. There are many beneficial ones like the honey bee, silk worm, etc. and quite a few that are harmful and cause direct or indirect damage to the well being of human beings. Researchers have been continuously trying to find new ways to mitigate problems of harmful insects like crop pests and also to harness the potential of beneficial ones. In this regard, advances made in genetic engineering have enabled the genetic modification of insects for various purposes. Some of the potential applications of this lie in crop pest management, vector management in public health, production of medically important proteins and genetic improvement of beneficial insects like parasitoids, predators, silk worm and honey bee. The proposed release of genetically engineered insects is evoking serious debate among researchers and environmental groups on safety issues as is happening with transgenic plants and engineered microbes.**

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## How It All Began

Genetic transformation of insects that involves introduction of DNA from external sources was first tried on a scale-less mutant of the stored-grain pest, *Ephestia khuniella*[1], in 1965. Injection of wild-type DNA resulted in the production of adults with wing scales. The success was repeated in 1971, when the lost eye colour was restored in the eye mutants of the same species when administered with wild-type DNA. Initial attempts to genetically modify the fruit fly, *Drosophila melanogaster*, resulted in somatic transformations with extra chromosomal inheritance. In other words, though transformation produced an effect, it was transient and could not be transmitted to the next generation. True genetic transformation of *D. melanogaster* was achieved

[1] A Mediterranean flour moth

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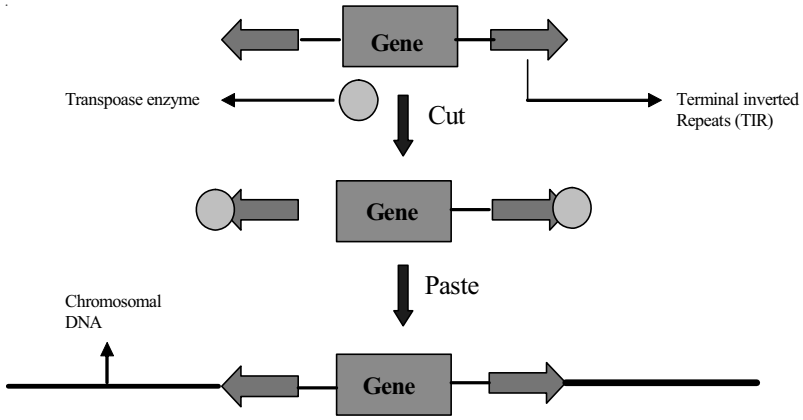
Genetic engineering, insects, applications.

The general mechanism of action of transposon is by cut and paste, which is aided by the transposon encoded enzyme called transpoase. In addition to the above, other mechanisms like replicative transposition, copy and paste are also common. Since transposons can move around the genome, they are called *jumping genes*.




Source:http://www.discoverygenomics.net

***Figure 1. Mechanism of transposon inserting at the target site.***

after the discovery of the transposon[2], P-element. (*Figure* 1). Later, the discovery of other transposons like *hermes, hobo, minos, mosI* and *piggyBac,* and novel DNA delivery systems such as microinjection[3], electroporation[4], and biolistics[5], accelerated work on genetic engineering of many agriculturally important insects for various purposes.

## Need for Genetic Engineering of Insects

Some insect species like mosquito and thrips are vectors of many dreaded human and plant diseases, respectively. These vectors are less amenable for conventional methods of control like spraying insecticides, which resulted in development of resistance and finally control failures. In this situation, genetic engineering of these vectors to make them refractory to the pathogens is a lucrative option. Similarly, some agriculturally important pests like cotton pink boll worm, *Pectinophora gossypiella* can be effectively managed by employing a technique called autocidal biological control (ABC) which would greatly reduce the insecticides usage and subsequent reduction in environmental pollution. In the same way, many desirable behavioural changes could be brought about in biological control agents and beneficial insects

[2] Transposons are segments of DNA that can move around to different positions in the genome of a single cell with the help an enzyme, transpoase.

[3] **Microinjection:** Refers to the process of using a micro needle to deliver substances into a living cell.

[4] **Electroporation:** An electrical treatment of cells that induces transient pores through which exogenous genetic material can enter the cell.

[5] **Biolistics:** A genetic engineering technique where particles are accelerated to deliver the genetic material directly into the cell.

within a short span of time, which otherwise take a long time using conventional method of breeding and selection.

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**How Genetic Modification of Insects is Carried Out**

**a) *Molecular Vehicle to Deliver the Gene of Interest***

Transposons are characterized by the presence of left and right terminal inverted repeats (TIR) and the gene of interest is placed between the TIR. For stable integration, two separate transposons, one carrying the gene of interest and a visible detectable marker within the functional TIR and another encoding a transpose with defective TIR are used. The most employed transposon is *piggy-back* which was discovered in insects that belonged to the order Lepidoptera. This transposon encodes a transpoase enzyme which has no similarity to any known eukaryotic transpoases. The advantage with this transposon is that no specific host factors are needed for transgenesis and hence results in stable transformation with high frequency. Use of other transposons often results in reduced frequency of transformation. In addition to transposons, several retroviral systems are also used to genetically engineer insects.

**b) Method of Transformation**

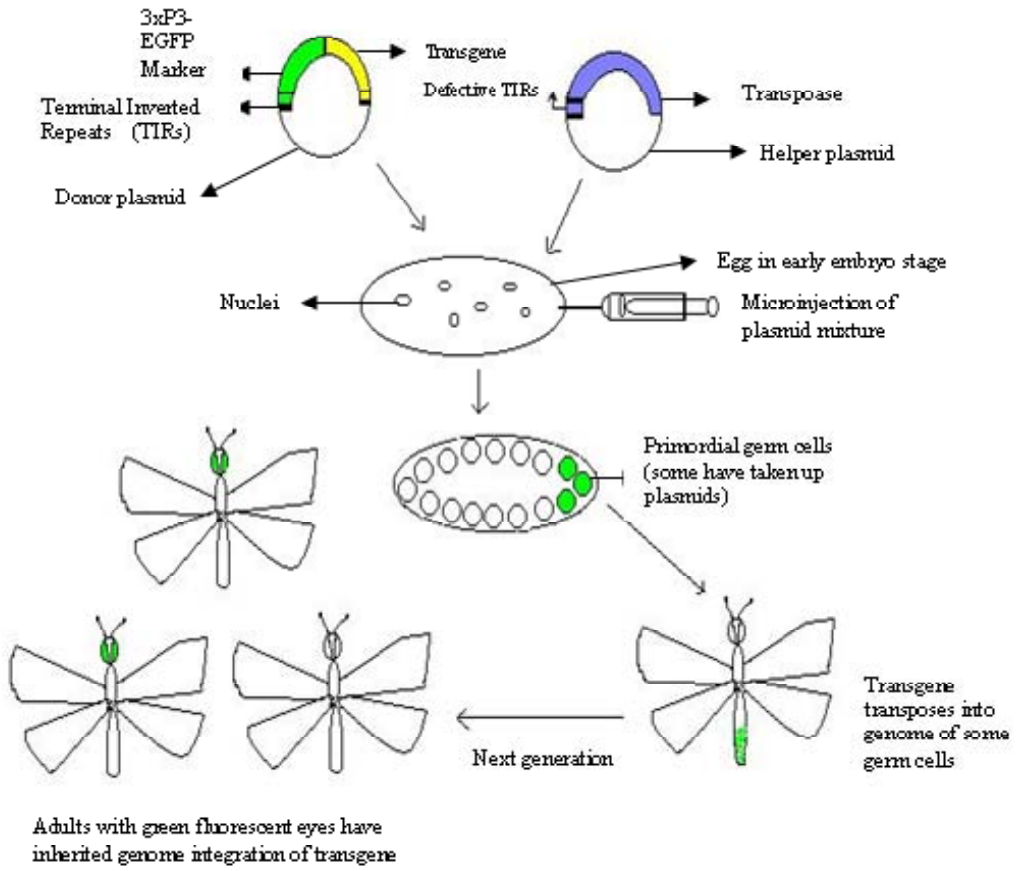
***Suitable developmental stage*:** Insects normally undergo four developmental stages viz. egg, larva, pupa and adult but variations are seen in some insects where they lack one of the above stages. Successful transformations have been achieved using the eggs, but adults have also been used though less frequently.

There are many methods available for delivering the gene of interest into the target species. The most common among them is microinjection, which requires a stereozoom microscope, a mechanical stage, micromanipulator and a mechanism for DNA injection (manual or electronic air-pulse system) (See *Box* 1).

There are many methods available for delivering the gene of interest into the target species.

The procedure involves aligning the needle with the micromanipulator and moving the mechanical stage to orient the micropyle

**Box 1. Genetic Engineering through Microinjection**




For genetic modification of insects, the transgene of interest has to be introduced into the germline of an egg and transposons are generally employed for delivering the gene of interest in insects. Among various transposons, *Hermes* element, *mariner* element *Mos1*, *Minos* and TTAA-specific element, *piggybac* are commonly used. For a stable integration of the transgene, two separate plasmids viz. donor plasmid, carrying the transgene of interest (yellow) and a visible transformation marker, the enhanced green fluorescent protein (EGFP) (green) driven by 3xP3 promoter, within the functional terminal inverted repeats (TIRs) and Helper plasmid that codes for a functional transpoase enzyme (violet) with defective TIRs are used. The Helper plasmid can not transpose itself as the TIRs are mutated. Mixture of two plasmids is then microinjected into the egg in the early embryo stage. During the primordial cell formation, some of the nuclei will take up the plasmids which result in the transposition of the transgene from the Donor plasmid onto a insect chromosome. Stable genetic transformation occurs, when the transgene is inherited in the next generation. The transformed insects could be easily selected by the expression of EGFP in eyes of the insects.

Source: Wimmer, 2003

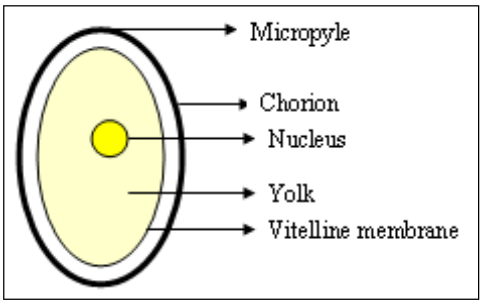
**Box 2. General Structure of an Insect Egg**

**Vitelline membrane:** The cell membrane of an insect egg is called vitelline membrane, which is a phospholipid bilayer that surrouds the contents of the egg cell.

**Cytoplasm:** The cytoplasm is distributed as thin band just inside the vitelline membrane. The nucleus of the egg cell lies within the yolk, usually close to one end of the eggs.

**Chorion:** In most insects a protective shell called chorion covers the egg. Chorion is perforated by many microscopic pores called aeropyles, which allow gaseous exchange without much loss of water.

**Micropyle:** It is a special opening near the anterior end of chorion, which allows the entry of the sperm into egg cell during fertilization.




end of the egg to the needle. The DNA carrying the gene of interest flanked by TIR and transpoase are delivered into the region of early embryos that contain germplasm. During embryonic development, the transpoase will mediate the transposition of the transgene onto a chromosome that results in the production of genetically modified insects. Modifications in microinjection technique are i) injection into ovarian egg follicles prior to oviposition and ii) injection into female haemocoel for uptake of DNA into egg follicles along with vitelline (*Box* 2).

The next most widely used method is lipofection[6] which is routinely used for DNA delivery into *in vitro* cultured cells. Other less common methods are i) biolistics which involves coating DNA with gold or tungsten microparticles and bombarding into cells or tissues, ii) electroporation which is currently employed to genetically engineer insects that belong to the orders Lepidoptera and Diptera.

[6] **Lipofection**: A process by which DNA or RNA which is encapsulated in an artificial phospholipid vesicle is delivered into eukaryotic cells.

In addition to the above direct methods, indirect methods like paratransgenesis[7] is also employed on insects which are less amenable for laboratory rearing or those that have a long generation time. In this method two bacterial endosymbionts (*Box* 3),

[7] **Paratransgenics:** Genetic alteration of microbes living in association with insects for various purposes.

**Box 3. Endosymbionts**

The term symbiosis originated from the Greek word *simbios*, elucidating a permanent association among different organisms and was first described by Anton de Bary in 1879. Among various symbiotic associations, endosymbiosis is unique, where a prokaryote is enslaved within a eukaryotic cell and is evident in 15 to 20 percent of insects. The symbionts are passed through generations by transovariole transfer. The host benefits from this obligate association by obtaining certain nutrients required for normal growth and development. Among various endosymbionts, two genera viz. *Wolbachia* (gram negative bacteria) and *Rhodococcus* (actinomycete) are important from pest management point of view. *Wolbachia* is predominant and is found in insects, nematodes, mites and spiders. *Wolbachia* infection results in diverse phenotypes of the host, ranging from induction of parthenogenesis, selective killing of males, altered sperm competition and cytoplasmic incompatibility. By genetic manipulation of a *Wolbachia* strain (*w*Cof), it is possible to drive a transgene of interest through a population for various purposes. The other endosymbiont, *Rhodococcus rhodnii* is found in the hindgut of the triatomine bug, *Rhodnius prolixus*, 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[8]. *R. prolixus* is made refractive to *T. cruzi* by genetically modifying *R. rhodnii* to express an antitrypanosomal peptide or a transmission-blocking antibody. Thus endosymbionts play a vital role in paratransgenesis.

[8] **Coprophagy:** Feeding or eating of dung or excrement that is a normal behavior among many insects, birds, and other animals

*Wolbachia* sp. and *Rhodococcus* sp. are commonly employed, though *Wolbachia* sp. is the most widely used for delivering genes to a whole population.

### c) Selection of Genetically Transformed Insects

The genetically engineered insects are selected using either NPT II (which confers resistance to neomycin analogues), or organophosphorus dehydrogenase (*opd*) (which confers resistance to paraoxan) or the gene for dieldrin resistance (*rdl*). Other useful visual markers are the green fluorescent protein (GFP)[9] and its spectral variants. For multiple transgene delivery, a reporter gene that is distinct from the marker is needed, where yellow and blue fluorescent proteins are very useful. The advantage with these markers is that they can show up in the live insect.

[9] **Green fluorescent protein:** A fluorescent protein that is found in a jellyfish that lives in the cold waters of north Pacific. The fluorescence is due to the protein acquorin that absorbs at the ultra violet radiation in the sunlight and emits it as low energy green light.

## Practical Utility of Genetically Modified Insects

The following are some of the uses of genetically engineered insects and many are summarized in *Box* 4.

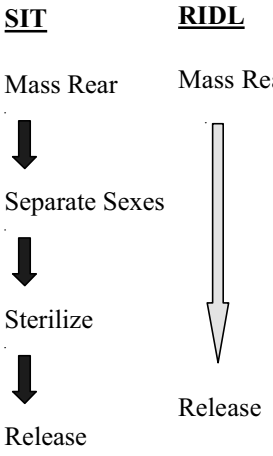
**Box 4. Some Genetically Engineered Insects and their Potential Applications**

| Insect | Potential applications |
|---|---|
| 1. Pink boll worm, *Pectinophora gossypiella* | * Autocidal biological control |
| 2. Mediterranean fruit fly, *Ceratitis capitata* | * Biased sex ratio toward male<br>* Pest eradication |
| 3. Phytoseiid mite, *Metaseiulus occidentalis* | * Biological control programme |
| 4. Nematode, *Heterorhabditis bacteriophora* | * Improved temperature tolerance |
| 5. Mosquito, *Anopheles*, *Aedes* | * Possible vaccine delivery system<br>* Interruption of virus life cycle to prevent multiplication and dissemination<br>* Long term changes in the blood feeding behaviour of mosquitoes |
| 6. Honey bee, *Apis* sp | * Improved yield of honey<br>* Enhanced pollination<br>* Resistance to viral and parasitic diseases |
| 7. Silk worm, *Bombyx mori* | * Improved silk properties<br>* Bioreactor for heterologous protein production<br>* Resistance to viral and parasitic diseases |
| 8. Fruit fly, *Drosophila melanogaster* | * Model organism for transformation studies |
| 9. Insect parasitoids and predators | * Increased egg laying<br>* Enhanced mass production<br>* Improve host searching<br>* Resistance to insecticides |

**i)** ***As bioreactors*:** Genetically engineered silkworms are employed as bioreactors for the production of the human skin protein, type III procollagen, which is used for covering wounds and in making artificial skin.

**ii)** ***Genetically Improved Biocontrol Agents:*** Insecticides not only kill pests but also many non-target, beneficial insects like parasitoids and predators. The conventional breeding and artificial selection for pesticide resistant natural enemies take many generations. But efforts are on to genetically engineer parasitoids and predators for general environmental hardiness, increased fecundity, improved host-seeking ability, etc.

**Box 5. Comparison of Sterile Insect Technique (SIT) and Release of Insects Carrying a Dominant Lethal (RIDL)**




**iii)** ***Impairing Disease Transmission:*** Insects, especially mosquitoes, spread a number of human diseases like malaria, yellow fever and viral encephalitis. These diseases are responsible for several million deaths each year and cause great losses to national economies. One genetic approach is to make mosquitoes unable to transmit diseases where it may be possible to develop resistant mosquitoes to over-express genes involved in neutralizing/encapsulating parastites in the insect stomach or salivary glands. In 1998, scientists successfully engineered the mosquito, *Aedes aegypti*, (which transmits yellow fever and dengue) using a non-pathogenic virus that contained a gene for preventing the dengue virus replication in salivary glands of *A. aegypti*.

**iv)** ***Insect pest Management****:* A species-specific approach to control insect pests is known as the sterile insect technique (SIT). But this method was largely unsuccessful and cost prohibitive in many agriculturally important pest species. In this direction, Thomas *et al*. (2000) made an improvement over SIT that is known as 'release of insects carrying a dominant lethal' (RIDL) (*Boxes* 5 and 6). The advantages of RIDL over SIT are listed in *Box* 7. In RIDL, there is scope for introducing three different types of traits into the insect genome.

1) Introduction of fluorescent transformation marker to easily identify the released insects for various ecological investigations.

2) Sex separation based on the female specific expression of a conditional dominant lethal gene. This facilitates release of males only in the SIT programme.

3) Embryo specific lethality after transmission to the progeny that could replace the harmful irradiation procedure and allow generation of competitive but sterile insects, which can be released at any stage of the life cycle.

The reserach team of Thomas Miller at the University of California, Riverside, USA is currently working on management of the pink boll worm, *Pectinophora gossypiella* on cotton, by releasing

**Box 6. Release of Insects Carrying a Dominant Lethal (RIDL)**

Sterile insect technique (SIT) involves release of irradiated males into the wild for pest management. This technique requires elimination of females as they do not contribute to the control. To achieve this various methods like mechanical sex separation methods using pupal mass, time of adult emergence etc are employed without satisfactory results. In addition to the above various female killing and genetic sexing mechanisms are employed where induced chromosomal aberrations affect the fitness of the insects.

Alternatively, an ingenious approach called 'release of insects carrying a dominant lethal' (RIDL) was demonstrated in *Drosophila melanogaster* by Thomas *et al*. (2000). In this method a transcriptional control element was used to derive the expression of the antibiotic, tetracycline repressible transactivator fusion protein, tTa. In the absence of tetracycline, tTa will derive the expression of any gene controlled by the tetracycline repressive element, tRe. For the purpose of eliminating females, tTa was first expressed under the control of fat body enhancer, Yp3 which is only expressed in females and not in males and a cytotoxic gene, Ras $64B^{V12}$ was expressed under tRe. The flies homozygous for Yp3-tTa were crossed with flies homozygous for tRe- Ras $64B^{V12}$ and reared on media with or without tetracycline. Normal sex ratio was observed on media containing tetracycline and no female progeny was produced on media without tetracycline. When the resulting transgenic males were mated to non transgenic females, no female progeny was produced which satisfy the requirement of RIDL. This approach holds a great promise in ecofriendly way of pest management as yolk proteins are expressed in a similar pattern in other insects that include agriculturally important insect pests.

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.

Possible risks in releasing genetically modified insects into the

**Box 7. Advantages of RIDL**

- No need to separate the sexes before sterilization
- No need to sterilize insects before release
- Female specific lethality can be achieved
- Accidental releases would pose no safety problem (RIDL stock would produce no viable progeny under normal environmental conditions)
- Releases can be made at any life-cycle stage of the target pest

**Box 8. Genome Size of Some Important Insects**

| Insect | Genome Size (Mbp) | No of Genes fully/partially sequenced |
|---|---|---|
| Fruit fly, *Drosophila melanogaster* | 170 | ~ 2000 |
| Med fly, *Ceratitis capitata* | 250 | ~ 40 |
| Mosquito, *Aedes* sp | 800 – 1500 | ~ 40 |
| Mosquito, *Anopheles* sp | 250 | ~ 100 |
| Silk worm, *Bombyx mori* | 530 | ~ 250 |
| Honey bee, *Apis* sp | 180 | ~ 50 |

Mbp – Million base pairs

environment may be:

- Disturbance of ecological balance.
- Total elimination of a pest species that give rise to another species to fill the vacuum.
- Viral vectors combining with other wild type viruses and also with the genome of the host.
- Exchange of transposons between organisms.

## Conclusions

Genetic engineering of different species of insects for various purposes is at a very nascent stage. The major bottlenecks are limited knowledge on molecular genomics of different species (*Box* 8), low frequency of transformation, high cost etc. Once these are overcome, genetic engineering of insects would be a job as routine as that of transformation of plants and microbes. Genetically engineered insects offer great scope for crop pest management, which would eventually result in reduced usage of pesticides. In addition, there is a possibility to modify behaviour of insects, improvement of efficiency of parasitoids and predators in biological control, disease and vector control in public health and vector management in plant disease management. One must however, bear in mind the possible negative impact of transgenic insect technology and its necessary fine-tuning. Till then, it is a long way to go before we harness the full potential of genetically engineered insects.

## Suggested Reading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