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GENETIC ENGINEERING OF INSECTS

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Introduction:

Genetic transformation of insects that involves information of DNA from external sources was first tried on a scale-less mutant of the stored grain pest, *Ephestia khuniella* 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 same species when administered with wild-type DNA.

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 the development of resistance and finally control failure. 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 bollworm, *Pectinophora gossypiella* can be effectively managed by employing a technique called autocidal biological control (ABC) which could greatly reduce insecticide usage and subsequently a reduction in environmental pollution. In the same way, many desirable behavioral changes could be brought about in biological control agents and beneficial insects witha short span of time, which otherwise take a long time using the conventional method of breeding and selection.

How the Genetic Modification of insects is carried out:

1) 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.

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The most employed transposons are piggyback which was discovered in insects that belonged to the order Lepidoptera. This transposon encodes a transferase enzyme which has no similarity to any known eukaryotic transposases. The advantages with this transposon are that no specific host factors are needed for transgenesis and have resulted in stable transformation with high frequency.

2) Method of Transformation:

Suitable developmental stage:

Insects normally undergo four developmental stages via, eggs, 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 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 stereo zoom microscope, a mechanical stage, a micromanipulator and a mechanism for DNA injection.

The process involves aligning the needle with the micromanipulator and moving the mechanical stage to orient the micropyle end of the egg to the needle. The DNA carrying the gene of interest flanked by TIR and transpose are delivered into the region of early embryos that contain germplasm. During embryonic development, the transpose will mediate the transposition of the transpose onto a chromosome that results in the production of genetically modified insects.

Modifications in microinjection techniques are:

1. Injection into ovarian egg follicles prior to oviposition.
2. Injection into female haemocoel for uptake of DNA into egg follicles along with vitelline.

The next most widely used method is **lipofection** which is routinely used for DNA delivery into *invitro* cultured cells. Other less common methods are:

i) **Biolistics** involves coating DNA with gold or tungsten microparticles and bombarding into cell tissues.

ii) **Electroporation** is currently employed to genetically engineer insects that belong to the order Lepidoptera and Diptera.

In addition to the above direct methods, indirect methods like "Paratransgenesis" are also employed on insects which are less amenable for laboratory rearing or those that have a

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long generation time. In this method, two bacterial endosymbionts *wolbachia sp* and *Rhodococcus sp* are commonly employed.

3. 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 paraoxon) or the gene for dieldrin resistance (rdl). Other useful visual markers are the green fluorescent protein (GFP) and its special variants.

Practical Utility of Genetically Modified Insects:

These are some practical utility of genetically modified insects:

1. 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.

2. Genetically Improved Biocontrol Agents:

Insecticides not only kill pests but also many nont arget beneficial insects like parasitoids and predators. The conventional breeding and artificial selection for pesticide resistant natural enemies take many generations. But efforts are on genetically engineer parasitoids and predators for general environmental hardiness, increased fecundity, improve host-seeking ability, etc.

3. Impairing Disease Transmission:

Insects especially mosquitoes, spread many 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 parasite in the insect stomach or salivary gland. In 1998, scientists successfully engineered the mosquito, *Aedes aegypti* using a non-pathogenic virus that contained a gene for preventing the dengue virus replication in salivary glands of *A. aegypti.*

4. 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

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agriculturally important pest species. In this direction, Thomas et al (2000) made an improvement over SIT that is known as the "release of insect carrying a dominant lethal (RIDL)." The advantages of RIDL over SIT are as:

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