GENETICALLY ENGINEERED, EMBRYO-SPECIFIC LETHALITY
FOR INSECT PEST MANAGEMENT

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Many insects compete with us humans for agricultural resources, feed on us, or act as disease vectors. Current control efforts rely mostly on the use of insecticides. The use of chemicals to either repel or kill insect pests is the oldest and most commonly used method of pest control. However, with the massive use of insecticides four major problems have arisen. First, many pests have developed resistance to one or several of the chemicals. Second, the non-specific action of these chemicals results in the destruction of beneficial animals, which has frequently led to ecological backlash phenomena with the rise of insecticide-resistant pests in large numbers. Third, the costs of developing new chemical products to overcome the problem of insecticide resistance are escalating. Fourth, the potential health hazard of many pesticides is a general threat to human welfare. Thus, novel and improved strategies are necessary to combat insect pests.

Genetic control based on the sterile insect technique (SIT) uses the release of mass-reared and sterilized insects to cause infertile matings that reduce the pest population level. The concept of fighting insect pests by a repeated inundation with sterile individuals of its own kind was already promoted in the first half of last century. The power of the technique lies in the simplicity of the biological principle on which it is founded and the lack of negative ecological effects following its application. Due to its species specificity, SIT is considered an environmentally friendly alternative to insecticides and has been successfully employed in area-wide approaches to suppress or eradicate pest insects like the pink bollworm *Pectinophora gossypiella* in California, the tsetse fly *Glossina austeni* in Zanzibar, the new world screwworm *Cochliomyia hominivorax* in North and Central America, and several tephritid fruit fly species in various regions of the world.

Additional programs indicate that these approaches would be valuable for a much more widespread use, but conventional sterilization by ionizing radiation decreases the competitiveness of sterilized insects. Thus, high quantities are required to inundate the pest population. This is a problem especially in lepidopteran pest species, where even highly irradiated males can still produce viable progeny. Moreover, SIT releases often require only males, but both sexes are needed for the rearing process. However, not only is it expensive to rear large numbers of potentially ‘useless’ females, but it is detrimental to release any females, sterile or not, in the case of species that sting fruit with ovipositors or transmit diseases by biting domestic animals and humans. In addition, for the Mediterranean fruit fly (medfly), *Ceratitis capitata*, male-only releases have been shown to increase effectiveness of the SIT.

Recent advances in insect transgenesis have encouraged the idea of transgenically manipulating pest insects in a way that will improve SIT approaches and widen its applicability. At least three different traits could be transgenetically introduced into insect strains to improve their use in the SIT: first, a marker gene could enable discrimination of released and naturally occurring insects; second, a female-specific lethality gene could allow for efficient genetic sexing; and third, a gene that causes lethality after transmission to the progeny could replace the irradiation procedure.

**Transgenic marking.** Discrimination between released sterile and wild insects is critical for monitoring the effectiveness of an ongoing SIT program. Currently, released insects are labeled with a fluorescent dye powder, which is expensive, labor intensive, and error-prone. The transgenic introduction of a fluorescent transformation marker, which does not compromise survival or fitness, would enable the identification of released insects in a simple way.

**Sexing strains.** In the medfly, separation of undesirable females has so far been based on genetic sexing strains that cannot be transferred to other species. Recently, transgene-based methods for sex-separation that are based on the female-specific expression of a conditional dominant lethal gene have been examined in the model insect...
Drosophila melanogaster and might be transferable to other insect pest species. In both studies, conditionality of female lethality was established by the use of the binary expression system based on the tetracycline-controlled transactivator (tTA), which can be suppressed by supplementing the food with tetracycline during insect rearing.

Replacement of irradiation. Currently, ionizing radiation is used to cause the required genetic damage that results in the death of sired progeny and thereby causes sterility. However, in medflies irradiation reduces the mating competitiveness by approximately 30%, and many lepidopteran pest species require especially high doses of irradiation, which impairs fitness severely. In order to generate sterile but vigorous insects, my Ph.D. student, Carsten Horn, followed a strategy that interferes neither with the adult phase of the insect life cycle nor with gametogenesis. The sterility is based on the transmission of a transgene combination that causes dominant embryo-specific lethality. This allows for the generation of vigorous and potent sterile insects, with males being able to transfer competitive sperm. The embryonic lethality is caused by the expression of a lethal factor under the control of a promoter that is active at early blastoderm stages only. If the male has been homozygous for the transgene combination, each fertilization event will lead to embryonic lethality. The advantage of this approach lies in the proposed high competitiveness of such males, since none of their reproductive organs is affected and matings actually lead to sperm transfer. For this, it is very important that the promoter is active only in early stages of development. Then the lethal phase can be passed while growing up under permissive conditions in the rearing facilities, whereas after release, non-permissive conditions will not affect the males themselves but only their progeny.

To cause organismal lethality, the proapoptotic gene head involution defective (hid) was chosen as effector gene, which induces cell death when expressed ectopically. To avoid down-regulation of HID, the phospho-acceptor-site mutant allele hidAla was employed. In order to limit the detrimental effect of the transgenes to the embryo, enhancer/promoters of genes that encode structural components of the microfilament network specifically required for blastoderm cellularization were used, such as serendipity a and nullo, which are absolutely specific to the blastoderm stage but are expressed then at very high levels. To establish conditionality of the embryonic lethality we also employed the suppressible binary expression system based on the tetracycline-controlled transactivator tTA.

The system was successfully tested in D. melanogaster; the lethality occurred efficiently and was restricted to the embryo. The progeny died as embryos and only rarely (1:10,000) did a larva escape. This is important, because for many insects, the larvae cause most of the economic damage. In laboratory experiments, the competitiveness of the male flies was not greatly affected by the transgene combination when a nine-fold excess of sterile males was used in competitive matings. Correspondingly, the progeny was reduced by about 85% in this situation.

Since broad range transposon vectors and widely applicable transformation markers were employed, the examination of this system should be straightforward in pest species for which germ line transformation protocols have been established. Currently, my group is testing to see if the embryonic lethality system can be combined with a female lethality system, which would then make it possible to raise vigorous but sterile males only.

Nevertheless, before thinking of any applications involving the release of transgenic insects, great care must to be taken to employ as many safety mechanisms as possible to prevent an undesired spread of the transgenes. Laboratory studies will first have to assess transgene stability and fitness constraints in large populations. To achieve transgene stability, transposons should be non-autonomous and chosen so that no endogenous or related transposon activities are present in the species of choice. To further avoid rare cross-mobilization of the introduced transgenes, vectors that enable effective immobilization by deletion or rearrangement of transposon ends should be developed. Moreover, the introduced transgenes must not contain positively-selectable drug resistance markers.
For first evaluations of the environmental impact of transgenic insects, SIT programs will minimize the ecological concerns that the release of transgenic organisms might bring about. The sterility of the released insects will serve as a biological safety mechanism that impedes vertical transmission of the transgenes, which will be removed from the ecosystems with the cessation of the SIT program. Transgene constructs containing fluorescent transformation markers only will be suitable for first field trials, since they will improve SIT applications by simplifying the monitoring, but should not provide advantages to the carrier organism and actually allow the identification of carriers at later stages. This will minimize the risk of a rare but potential horizontal gene transfer. Given the understandably intense public scrutiny and the general lack of knowledge on potential risks, all projects that require the release of transgenic insects into the environment need to be planned with utmost care. Already at the initial stages of this methodology, molecular and population geneticists, entomologists, ecologists, as well as pest management specialists need to coordinate their efforts along with regulatory agencies to establish a safe use of the great potential transgenic insects have to offer.

References


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