**Transgenic Spodoptera exigua: possibilities for their use**

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Transgenic *Spodoptera exigua* are being developed by microinjection of a piggyBac vector. The vector expresses green fluorescent protein (GFP) under the control of the actin promoter. Forty percent of the first-instar larvae that hatched from the injected eggs was green fluorescent. However, after backcrossing none of the G1 first-instar larvae was fluorescent and a transgenic line could not be established. Several possibilities for the use of transgenic insects are discussed.

**Keywords:** genetic engineering, pest management, piggyBac, microarray

The ability to genetically engineer arthropods using recombinant DNA methods opens new opportunities for improving pest management programs. For this reason we started transformation of *Spodoptera exigua* (beet armyworm) as a model for moth transformation. This nothiid moth species is a serious pest of ornamental and vegetable crops in greenhouses and shows tolerance and resistance to a number of chemical insecticides.

The piggyBac transposase gene can be used for transformation of insects in different orders: the fruit fly, *Drosophila melanogaster*, the flour beetle, *Tribolium castaneum* and recently the silk moth, *Bombyx mori*. In this paper we report the use of piggyBac for transformation of *S. exigua*.

**MATERIAL AND METHODS**

The vector pPIGA3GFP without a transposase but with long terminal repeats (LTRs) for recognition by the piggyBac transposase and the disabled vector pH32PIG carrying the piggyBac transposase but without the LTRs for the generation of stable, non-transposing germ lines were obtained from Dr. P. Couble (Lyon). The plasmids are described by Tamura *et al.* (2000). The transposated element expresses green fluorescent protein (GFP) under the control of the actin promoter.

*S. exigua* was cultured as described by Smits *et al.* (1987). The day-night rhythm was reversed, because eggs are only laid in the dark period. *S. exigua* moths were allowed to lay eggs for one hour on a piece of paper. Subsequently the egg-paper was removed from the container and egg clutches were cut out of the paper. Adult abdominal hairs, which are laid across the clutches by depositing females, were removed with a brush. The eggs were stuck on a piece of Removable Magic tape (Scotch 811) and the paper was removed. Now the posterior end of the egg is facing up. A small drop (1/10 of the size of the egg) of a 1:1 mixture of vector and helper plasmids (0.4 µg/µl total DNA concentration) in water, was injected into the posterior end of each egg using a FemtoJet injector (Eppendorf). Embryos were allowed to develop at 25°C.

After 4 days the larvae hatched and the fluorescence of the GFP was observed under a microscope equipped for epifluorescence detection. Filters passing light between 507 and 510 nm and between 450 and 480 nm were used for detection and excitation respectively. Both fluorescent and non-fluorescent larvae were transferred to artificial diet and reared under standard conditions. G1 adults were backcrossed to moths of the recipient strain and their offspring was again tested for GFP expression.

**RESULTS**

About 3500 eggs were injected and 10% of these eggs hatched. Of the 350 first-instar larvae that hatched from the injected eggs, 40% was green fluorescent (Fig. 1). However, after backcrossing none of the G1 first-instar larva was fluorescent and a transgenic line could not be established.
DISCUSSION

Transgenic S. exigua

The transformation method used here did not generate germ-line transformation of *S. exigua* with reasonable frequency. This could have several reasons. Firstly, the *piggyBac* vector may not be suitable as a vector for *S. exigua* transformation. In that case the green fluorescence observed in the larvae after injection, is not the result of expression by transformed somatic cells, but the result of transient expression derived from injected vector DNA still present in the somatic cells. This may be checked by Southern blot analysis. If the *piggyBac* vector is indeed not suitable, other vectors, like *Hermes* or *mariner*, may give better results.

Secondly, it may be that the position of the injection in the egg is not close enough to the spot where the germ cell formation takes place. Within the egg, diffusion of injected DNA is very limited. When the DNA is injected in the wrong part the transposable element with the GFP gene will not be incorporated in the germ-line cells and, therefore, no germ-line transformants will be generated. Although, in general, germ cells are formed in the posterior end of the eggs, there are differences between insect species. For instance, in *B. mori* germ cells are formed at the ventral side of the egg. Cytological studies on germ cell formation may reveal the best site for injection of *S. exigua* eggs.

Thirdly, it is also possible that the survival of the germ-line transformants is so low that the number of eggs injected is not sufficient to find one germ-line transformant (less than 0.03%). The addition of purified transposase might improve the percentage of germ-line transformants (Coates *et al.*, 2000). However, even a tenfold improvement would not be enough to make this a useful transformation system.

Possibilities for the use of transgenic insects

In Europe public opinion is against the release of transgenic organisms in nature. However, the use of transgenic organisms in contained units is still feasible and opens a range of opportunities. Transgenic insects can be used for research purposes but can also be of practical use in, for instance, plant breeding. The following research opportunities are accessible with transgenic insects.

Gene knock-out/ knock-in to study gene function

To study the function of a gene, a gene can be knocked-out to see what changes occur in the insect. Genes can be efficiently silenced using the RNAi approach (double stranded RNA). The other way around, a gene can be added (knock-in) to an insect-line that lacks the gene or a gene can be overexpressed.
**Figure 2.** Preparation of GFP-insects for high throughput screening. Gene expression, which is induced by toxins or resistant plants is identified by DNA microarrays. The promoter of such a gene is used to regulate GFP expression in transgenic insects. These transgenic insects are green fluorescent on a resistant plant or after feeding on toxins.

**Differentiation between populations in population dynamics studies**

The study of insect behaviour and ecology could benefit greatly from marker insects in order to estimate population size and flow. The transgenic insect population will have an easily scorable phenotype like GFP, eye color etc. and can be distinguished from other insect populations.

**Screening for toxins and insect resistance in plant breeding**

Most plant resistance traits against insects are based on factors, which are not immediately toxic and cause only a partial level of resistance. The measurement of these resistance effects using conventional parameters such as insect survival, developmental time, and fertility are difficult and extremely costly in terms of labour. One can assume that moderate effects on insect reproductive success are induced by significant, immediate and detectable changes in insect gene expression. Changes in gene expression will, therefore, most likely more easily monitor plant resistance to insects than derived traits such as survival, developmental time, and fertility. Genes that are specifically expressed when an insect is feeding on a resistant plant, but not a susceptible plant, can be identified by DNA microarrays. The promoter of such a gene can be used to regulate GFP expression in transgenic insects (Fig. 2). These transgenic insects are green fluorescent on a resistant plant but not on a susceptible plant and can be used for high throughput screening of plant resistance. Similarly, promoters of genes that are specifically expressed when an insect eats a toxin, can be used for high throughput screening of new insect toxins.

**REFERENCES**

