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RESEARCH

Gene Transfer into Corn Earworm (*Helicoverpa zea*) Embryos

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Transposable elements with short inverted repeats at their termini have been identified in a number of diverse insect species and have proven to be useful gene delivery vectors for the transformation of *Drosophila melanogaster*. In this report we examine the ability of the *D. melanogaster hobo* element to transpose in lepidopteran species. A *Trichoplusia ni* (cabbage looper) and a *Helicoverpa zea* (corn earworm) embryonic cell line were found to be capable of supporting productive transposition of the *hobo* element as measured by a plasmid-based excision assay. Furthermore, *hobo* transposition was detected in *H. zea* embryos in a manner consistent with that seen for the cell line. In both cases, transposition/excision was found to be independent of vector-encoded transposase functions, indicating that endogenous genes are involved in *hobo* mobility. Finally, we demonstrate the stable insertion of the bacterial *lacZ* gene into the *H. zea* genome. These data demonstrate that *hobo* elements are capable of transgressing species boundaries and functioning in non-drosophilid cellular environments. More importantly, this represents the first description of a genetic transformation system for a lepidopteran species.

The tobacco budworm (*Heliothis virescens*) and the corn earworm (*Helicoverpa zea*) are pests on a wide variety of crops including cotton, corn, soybean, and other economic and ornamental plants (Mitter et al. 1993). To date, their control has been unsuccessful and has been attempted almost exclusively through the use of synthetic organic pesticides (Bull and Menn 1990). This clearly underscores the need for the development of safe and effective alternatives to chemical insecticides and has stimulated significant interest in using genetically engineered insects as biological control agents. However, the use of such an approach is limited by the lack of techniques available to manipulate insect genomes.

There is an enormous need for gene vectors and techniques that will allow for the transformation of any non-drosophilid insects. To date, *Drosophila melanogaster* is the only insect species that supports routine genetic transfer (Heilman et al. 1993). In the absence of this technology, the full repertoire of molecular genetic tools and techniques cannot be applied fully to the problems of insect biology that continue to affect world health and agriculture. The ability to orchestrate genotypes is critical for our comprehension of a multitude of pest problems, including the rapid emergence of insecticide resistance (Georghiou 1986; Pimental et al. 1990) and the complexities of the *Plasmodium/Anopheles* interaction (Bryce et al. 1994; Gwadz 1994). In addition to understanding the biology of complex associations, current and future pest control measures could benefit considerably from the availability of tools for manipulating insect genomes. Presently, only the sterile insect release method has been used extensively and successfully. This involves the release of sterile males (sterilized with radiation) in sufficient numbers to complete successfully with resident males to mate with native females (Knipling 1979; LaChance 1979; Lewis 1993). Numerous alternative control strategies have been discussed, including hybrid sterility, sex-linked recessive lethal mutations, as well as conditional dominant lethal mutations (DeVault et al. 1996; Heilmann et al. 1994). The availability of a "broad host range" insect transformation system will provide the tools necessary to address some of these problematic questions.

Transposable elements are structurally di-

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verse and can be divided into several groups, based on their mode of transposition, DNA sequence similarity, and open reading frame (ORF) gene order. Once such family of elements, the short inverted repeat element family, has been shown to be very amenable to in vitro manipulation. Members of this family have several features in common, including short inverted repeats with somewhat weak sequence similarity, and 8-bp target-site DNA duplications. In addition, Calvi et al. (1991) have found that the polypeptide sequences of the putative transposases of three very distinct members of this family [Activator (Ac) from *Zea mays,* Tam3 from *Antirrhinum majus,* and *hobo* from *D. melanogaster*] have several regions of significant amino-acid sequence similarity and homology, suggestive of a horizontal gene transfer event between the plant and animal kingdoms. We have identified previously *hobo*-like DNA sequences in the genomes of both *H. zea* and *H. virescens* (DeVault and Narang 1994), suggesting that the *hobo* element may function in these backgrounds. In addition, *hobo* elements have been used as gene delivery vectors to transform *D. melanogaster* (Blackman et al. 1989). Therefore, in this study we examine the transposition ability of the *hobo* element in lepidopteran cellular and embryonic environments. The results from this study clearly indicate that the *D. melanogaster hobo* element is functional in *H. zea* and can be used to deliver foreign genes to the heterologous genome.

RESULTS

hobo Transposition in Lepidopteran Cell Lines

A method for analyzing the ability of the *D. melanogaster hobo* transposon to excise from an introduced plasmid in heterologous insect species has been described previously (Atkinson et al. 1993; Leopold et al. 1996). In this study, this system is extended to two lepidopteran cell lines (Fig. 1; Table 1). The basis of the assay is that if the element is functional (capable of mobility) in the non-host background, it could be detected easily by analyzing for excision of the element from the introduced plasmid. Table 1 clearly demonstrates that the *hobo* element was readily excised from the reporter plasmid in both *H. zea* (0.219% at 25°C) and *Trichoplusia ni* (0.053% at 25°C) cell lines. Particularly interesting was the observation that excision of the *hobo* element from the assay plasmid was significantly increased (136.5% for *H. zea* and 318.9% for *T. ni*) following a 4-hr heat shock. It should be noted that this excision is in the absence of an introduced functional *hobo* transposase. To analyze this latter effect in greater detail, plasmid pHSH2 and the reporter plasmids (pHEB4 and pBR–*lacZ*) were cotransfected into recipient cells, and the excision assay performed. Plasmid pHSH2 harbors the *hobo*-transposase ORF under the control of the *D. melanogaster* heat shock promoter (*hsp72*), a promoter demonstrated previously to be functional in lepidopteran backgrounds (K.J. Hughes, S.K. Narang, O.A. Johnson, R.A. Leopold, and J.D. DeVault, in prep.). Table 2 clearly shows that there is no real increase in the level of excision when the exogenous transposase function is provided. The frequency of the excision event is sig-

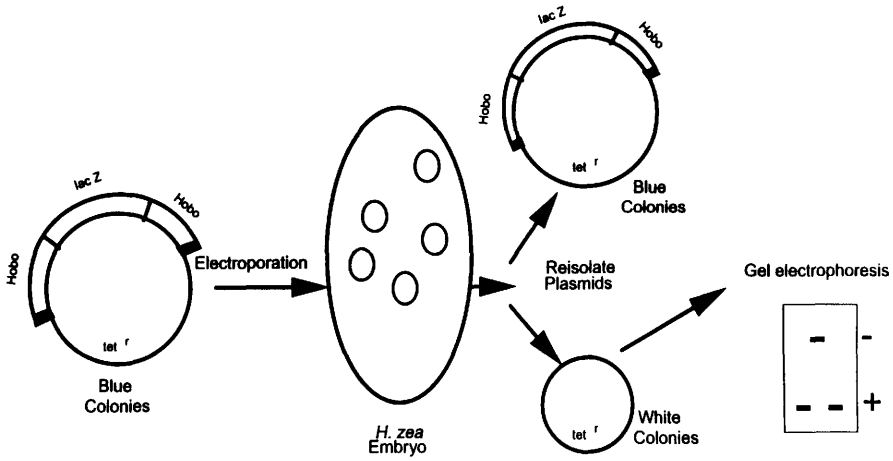

Figure 1 The *hobo* excision assay. Plasmid DNA was introduced into early blastoderm embryos of the corn earworm using electroporation, and into cell lines with lipofectamine reagent (GIBCO/BRL). Excision of the *hobo* transposon was analyzed by isolating plasmid DNA from manipulated embryos and cell cultures, and transforming *lacZ*$^-$ *E. coli* strain JM109. Initial positive excision events were identified by the appearance of white colonies following bacterial transformation. Successful excision was verified by evaluating disparity of plasmid products utilizing gel mobility analyses.

Table 1. Mobility of *hobo* Transposon in Lepidopteran Cell Lines

	H. zea (°C)		*T. ni* (°C)	
	25	**37**	**25**	**37**
Repetitions	12	12	10	10
Plasmids (no.)	4112	5023	3768	4964
lacZ deletions	9	26	2	11
Frequency[a]	0.219	0.518	0.053	0.222
Increase (%)	—	136.5	—	318.9

T. ni (Hi 5) and *H. zea* (HZAM1) cell lines were transfected with plasmid pHEB4 or plasmid pBR–lacZ following standard conditions for lipofectamine reagent (GIBCO/BRL). Following 4 hr at the specified temperature, the standard excision assay was performed.
[a]The number of rescued plasmids lacking the *lacZ* element per plasmids rescued per cell harvested.

nificantly reduced in both cell lines in the presence of introduced transposase. In contrast, when this construct is used in receptive strains of *D. melanogaster* (E−) a significant increase in the level of excision is observed (data not shown). The plasmid construct pHEB4 is a replacement plasmid whereby an internal portion of the coding region of the transposase ORF has been replaced by the *lacZ* gene. Therefore, these results suggest that endogenous *H. zea* and *T. ni hobo*-like transposase proteins are being induced in response to the environmental heat shock and providing the necessary functions for *hobo* transposition. It should be noted that when plasmid pBR–*lacZ* was substituted for plasmid pHEB4, no excision events were observed implicating the *hobo* element in the excision event.

hobo Transposition in *H. zea* Embryos

To extend these observations to the organism, the plasmid pHEB4 was electroporated into 6-hr-old *H. zea* embryos and DNA isolated as described in Methods, below. Table 3 confirms that the *D. melanogaster hobo* element is functional in post-gastrulation *H. zea* embryos. In the absence of an environmental heat shock, the frequency of *hobo* excision varied only slightly from 1.6%–2.0% of plasmids analyzed. However, in the presence of the heat stimulus, the occurrence of the excision event increased to a range from 5.4%–9.7%. This is in sharp contrast to the results obtained from co-introducing plasmid pHSH2. Table 4 reiterates the data reported in Table 2. In the presence of the helper plasmid pHSH2, there was actually a decrease in the level of the observed excision events. Again, when the control plasmid pBR–*lacZ* was used as the reporter construct, excision was never observed. These results support the contention that the *hobo* element may be useful as a gene delivery vector, and represent the first description of transposon mobility in a lepidopteran embryo.

Table 2. Mobility of *hobo* Transposon in Lepidopteran Cell Lines in the Presence of Helper Transposase

	H. zea (°C)		*T. ni* (°C)	
	25	**37**	**25**	**37**
Repetitions	12	12	10	10
Plasmids (no.)	5,098	6,657	4,135	5,879
lacZ deletions	7	18	2	5
Frequency	0.137	0.270	0.048	0.085
Increase (%)	—	97.1	—	77.1

Plsamids pHEB4 and pBR–lacZ were cotransfected with plasmid pHSH2 into both *H. zea* and *T. ni* cell lines and excision assay performed as described in Methods.

Table 3. Temperature Effect of *hobo* Excision in *H. zea* Embryos

Embryos	Transformants	Excised	Percent excised[a]
	25°C		
50	432	7	1.62
100	712	14	1.97
75	545	11	2.02
50	392	8	2.04
	37°C		
100	540	41	7.59
50	340	31	9.12
50	310	30	9.67
70	286	24	8.39

Embryos were electroporated with 8 μg of pHEB4 DNA per reaction, allowed to recover at room temperature for 18 hr, at which time they were placed at the designated temperature for a period of 4 hr. DNA was then isolated, and *E. coli* strain JM109 was transformed.
[a]The percentage of rescued plasmids lacking *lacZ* elements as determined by gel electrophoresis.

Construction of Transgenic *H. zea*

To examine the potential of using the *hobo* element to construct transgenic *H. zea*, embryos were harvested and treated exactly as described for the excision assay. However, instead of harvesting plasmids from the electroporated embryos, development was allowed to proceed, eggs allowed to hatch, and larvae permitted to mature to pupation. Presently, there is no useful marker for following gene transfer in a lepidopteran background. Therefore, on emergence of adults from the pupal stage, PCR of the individual pupal casings was performed (Fig. 2). It was found that out of a total of 1000 embryos subjected to electroporation, 17 (or 1.7%) adults harbored the *hobo–lacZ* insert.

To rule out the possibility that the observed *lacZ* element may in fact be the result of the electroporation of a bacterial species, three separate experiments were performed. First, 10 μl of DNA from each pupal casing was used to transform *Escherichia coli* strain JM109 (see Methods) and scored for by selection in the presence of tetracycline. No tetracycline-resistant bacteria were observed from any of the pupal casing samples, demonstrating that the observed *lacZ* elements were not the result of the electrotransformation of a bacterial species with intact plasmid DNA, nor the result of stable maintenance of the intact plasmid. To address the possibility that the amplified DNA was the result of the chromosomal insertion of plasmid pHEB4 into a bacterial chromosome (i.e., endosymbiont), each sample was subjected to PCR analyses with primers specific for both eukaryotic and prokaryotic ribosomal DNA (Turberville et al. 1991; O'Neil et al. 1992). In each instance, an appropriately sized amplified product was detected only with eukaryotic-specific primers and never with prokaryotic-specific primers (data not shown). Finally, pupal casings were rinsed extensively with Luria broth, and divided into two samples. One sample was placed at 30°C in a rotary incubator (aerobic environment) for 30 hr, whereas sample two was placed in an anaerobic chamber for a similar amount of time. These samples were then subjected to PCR analyses using all of the primers listed above with absolutely no detection of amplifiable DNA (data not shown), thereby confirming the absence of bacterial species associated with the pupal casings. These experiments conclusively demonstrate that the amplified *lacZ* elements are not the result of a bacterial artifact, bacterial transformation, or the result of maintenance of plasmid DNA. Furthermore, the fact that these elements were identified in pupal cas-

Table 4. Effect of Helper Transposase on *hobo* Excision in *H. zea* Embryos

Embryos	Transformants	Excised	Percent excised[a]
	25°C		
150	538	5	0.93
100	387	3	0.78
175	672	5	0.74
	37°C		
150	612	18	2.94
100	387	11	2.84
175	504	16	3.17

Embryos were electroporated as described with 8 μg of pHEB4 DNA and 8 μg of pHSH2 DNA per reaction, allowed to recover at room temperature for 18 hr, at which time they were placed at the designated temperature for a period of 4 hr. DNA was then isolated, and *E. coli* strain JM109 was transformed.
[a]The percentage of rescued plasmids lacking *lacZ* elements as determined by gel electrophoresis.

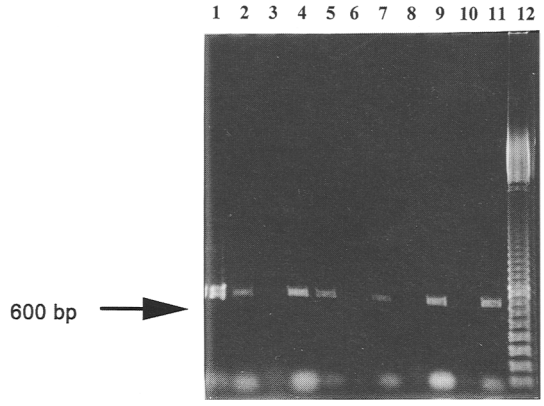

Figure 2 PCR analysis of parental pupal casings. DNA was extracted from pupal casings and subjected to PCR using bacterial *lacZ*-specific primers. This is a representation of the samples analyzed. (Lane *1*) Plasmid pHEB4; (lane *2*) plasmid pBR–*lacZ;* (lane *3*) nontreated pupal casing; (lanes *4–11*) representative samples; (lane *12*) 100-bp ladder (GIBCO/BRL).

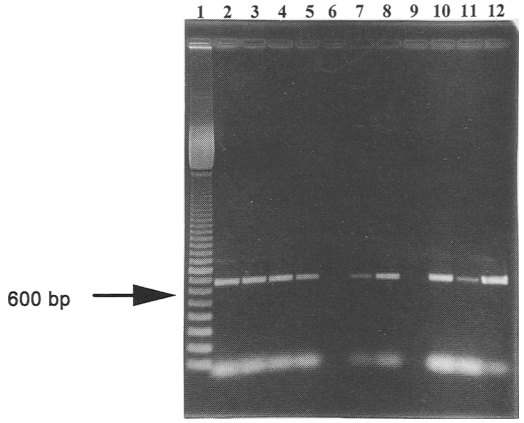

Figure 3 PCR analysis of pupal casings from progeny of wild-type males crossed to *lacZ*$^+$ females. (Lane *1*) 100-bp ladder; (lanes *2–12*) representative samples from the backcross described.

ings of an organism that has undergone complete metamorphoses suggested that the *lacZ* DNA sequences had transposed into the genome of the host insect. The sensitivity of this methodology is best exemplified by the fact that it is routinely used to amplify bacterial DNA from minute environmental samples (O'Neil et al. 1992).

To determine the genetic transmission of the inserted DNA, each transgenic adult was backcrossed to a wild type of the opposite sex, resulting progeny allowed to develop into adults, and pupal casings examined for the presence of the *lacZ* fragment (Fig. 3). We found that in each incidence, the *hobo–lacZ* element was vertically transmitted in an apparently stable manner. To quantitate the frequency of inheritance of the *lacZ* element, one female and one male were followed through several generations and inheritance patterns analyzed (Fig. 4). Because the nature of the PCR reaction is not quantitative but qualitative, the genetic scoring of the *lacZ* insert most closely approximates that used for a dominant marker. Table 5 presents data observed from following one female and one male through three generations. These data have, to date, been extended to five generations with similar results. The founders (P1) for each line consisted of one *lacZ*$^+$ adult and one wild-type *lacZ*$^-$ of the opposite sex. The F_1 generation (Table 5) represents the raw data obtained from two such crosses. A total of 231 progeny resulted from the positive male, whereas 197 progeny arose from the positive female. The corresponding ratios of positive to negative DNA samples closely approximates the 1:1 ratios that one would expect to observe when scoring a dominant visible phenotype. The corresponding 3:1 ratios observed in the F_2 progeny possibly suggest that the *lacZ* sequences are

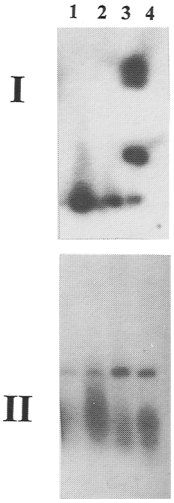

Figure 4 (*I*) Southern blot hybridization of genomic DNA from generation 5 adults. DNA was extracted, digested with *Sma*I, and subjected to Southern hybridization to the *lacZ* fragment. (Lane *1*) Female adult; (lane *2*) male adult; (lane *3*) 1-kb ladder (GIBCO/BRL); (lane *4*) no DNA. (*II*) Genomic DNA was amplified with *hobo*-end primers and subjected to Southern hybridization analysis with the *lacZ* fragment. (Lanes *1,2*) Male adult DNA; (lanes *3,4*) female adult DNA.

Table 5. Inheritance Pattern of *lacZ* Sequences

	P1 (male)			P2 (female)		
Generation	*lacZ*$^+$	*lacZ*$^-$	% *lacZ*$^+$	*lacZ*$^+$	*lacZ*$^-$	% *lacZ*$^+$
F_1	122	109	52.81	96	101	48.73
F_2	212	68	75.71	157	49	76.21
F_{3A}	238	0	100.0	264	0	100.0
F_{3B}	173	0	100.0	87	0	100.0
F_{3C}	116	35	76.82	74	21	77.89

Pupal casings were collected from newly emerged adult moths, DNA was extracted and subjected to amplification with PCR primers specific for the *lacZ* sequences. Three successive generations resulting from the P1 adult mated to a wild type of the opposite sex. Subsequent crosses were between *lacZ*$^+$ adults from the same genealogical line.

present in one copy, as a frequency of 1:1 or higher would be expected if the sequences were present in two or more copies. However, the possibility also exists that multiple copies are inserted in close proximity to one another. In either event, these data demonstrate the introduction of foreign DNA onto the chromosome of the corn earworm. To assess the structure of the integrated DNA, Southern blot hybridizations were performed on adults from generation 5 (Fig. 4I, II). If the transposition event was a singular event, hybridization of *Sma*I-digested genomic DNA with a *lacZ* probe should produce bands of 500 bp. This is the observation presented in Figure 4A. To rule out the possibility that the *lacZ* insertion was independent of the *hobo* element, PCR primers corresponding to terminal sequences of the *hobo* element were used to amplify genomic DNA. This DNA was then subjected to Southern hybridization to the *lacZ* fragment. Figure 4B clearly shows that PCR-amplified fragments are capable of hybridizing to the *lacZ* probe.

DISCUSSION

The development of a method for germ-line transformation is absolutely required for the molecular analyses of economically important insects, whether they be beneficial insects or agricultural pests (DeVault et al. 1996). Several obstacles need to be overcome to achieve this goal, including the technology for the delivery of foreign DNA to the chromosome of the target species and a means by which introduced DNA can be easily monitored for insertion and inheritance. To date, only *D. melanogaster* and the Mediterranean fruit fly (*Ceratitis capitata*) are capable of supporting successful genetic transformation (Rubin and Spradling 1982; Spradling and Rubin 1982; Loukeris et al. 1995). In both instances, phenotypic markers allow for the simple screening of transformants and charting of the introduced DNA through successive generations (Zwiebel et al. 1995). In this study, a method is described for the introduction and monitoring of foreign DNA into a lepidopteran insect pest in the absence of phenotypic markers.

This represents the first demonstration that the *D. melanogaster hobo* element is functional in a lepidopteran species. Specifically, it was shown that the *hobo* element can be mobilized in cell cultures of the corn earworm *H. zea,* and the cabbage looper *T. ni* (Tables 1 and 2). The measurement of mobilization in these two cell lines involved a simple genetic test whereby a plasmid harboring a manipulated *hobo* transposon containing a *lacZ* gene, was used as a reporter. Excision of the *hobo–lacZ* element was initially identified by the appearance of white colonies on MaConkey agar following transformation of *E. coli* strain JM109. Verification of the loss of the insert was subsequently accomplished by restriction analyses of isolated plasmids. It was found that excision was influenced by an environmental heat shock that presumably stimulates the production of an endogenous *hobo*-like transposase protein. If this is the case, additional (as of yet) unidentified means will have to be developed to guarantee stability of the insert. In the absence of further analyses of the excision sites present on the donor plasmid or of the fate of the excised DNA, the exact nature of the excision event cannot be resolved. Although it is speculative to as-

sign a causative effect of the *hobo* transposon on the observed excision the apparent inhibitory effect of introduced exogenous *hobo* transposase protein (pHSH2) suggests that the product of this introduced ORF has some, as of yet unidentified, role in the excision process.

When these results were extended to include *H. zea* embryos, similar results were consistently observed. Leopold et al. (1996) and K.J. Hughes, S.K. Narang, R.A. Leopold, O.A. Johnson, and J.D. DeVault (in prep.) have described elsewhere the applicable technology and parameters for the successful electroporation of corn earworm embryos. Using these parameters, it was found that not only was the *D. melanogaster hobo* element capable of excising in the heterologous background, but the observed excision frequency was generally 8- to 10-fold higher than that seen for the normal host species or other dipteran species using the same techniques (Leopold et al. 1996). The *hobo* element has been shown to be readily mobilized in *D. melanogaster,* sheep blowfly, and housefly (Atkinson et al. 1993; Calvi et al. 1994; O'Brochta et al. 1994; Handler and Gomez 1995; Leopold et al. 1996). This excision has been shown previously to be somewhat inhibited by the overproduction of the *hobo* transposase ORF. This could be the result of transposable immunity. Transposable immunity is a syndrome generally associated with bacterial systems, however, it is well known that the presence of a specific transposon (presumably its transposase) at a critically high-copy number prevents further mobilization by that element (Finnegan and Fawcett 1986). The identification of a comparable level of excision in *H. zea* suggests that the processes may be similar across species boundaries.

Two other members of this family of transposable elements, Ac and Tam3, have been shown to be fully functional in the genomes of a wide variety of alternative plant hosts (Wienand and Saedler 1988). Therefore, it is was of interest to determine if the observed high rate of *hobo* excision in the lepidopteran background would correspond to a measurable rate of transposition (i.e., excision from the donor plasmid and concomitant insertion into the genome of *H. zea*). A major hindrance for monitoring gene transfer into organisms other than *Drosophila* has been the lack of a scorable phenotypic marker. Those markers that were previously available require the sacrifice of the putatively transformed insect. To circumvent this obstacle, PCR analysis of the pupal casings of individually emerged moths was performed. It was felt that there would be enough epidermal and tracheal cells present in the pupal casing shed by the pupariating earworm to allow for PCR analysis using primers specific for the bacterial *lacZ* gene. These primers were selected over *hobo*-specific primers as *hobo*-like sequences have been identified previously within the genome of this species (DeVault and Narang 1994). This approach proved extremely successful in identifying those newly emerged adults harboring the *hobo–lacZ* with their genomes. This strategy allowed for the examination of all manipulated embryos without the need of sacrificing those that may contain the inserted sequence. It was found that it was possible to consistently generate transgenic moths at a frequency of 1.7% of those that were manipulated. The frequency of actual insertions approached 1.9% of embryos manipulated, however, under the conditions of our experiments, we chose not to include those pupae that produced adult moths that died before being able to mate.

CONCLUSIONS

The ability of the *D. melanogaster hobo* transposon to function in heterologous species will have tremendous potential and significance, as it will allow for the development of broad host range insect transformation vectors. Recently, other researchers have shown that using another transposable element as a delivery vector, stable transformation of the Mediterranean fruit fly is possible (Loukeris et al. 1995). These investigators were able to capitalize of the similarities between the Medfly and *Drosophila* for the identification of an easily scorable phenotypic marker (Zwiebel et al. 1995). It has been suggested that a unique transformation system will need to be developed on a species-by-species basis. It is our belief that the *D. melanogaster hobo* element will prove to be amenable to the introduction of foreign DNA to a number of diverse insect species. In this report we demonstrate this to be true for the corn earworm and studies are currently underway to determine this potential in other lepidopteran species.

METHODS

Plasmids, Bacteria, and Cell Lines

Plasmid pHEB4 was a gift from Dr. P. Atkinson (CSIRO, Canberra, Australia) and contains the α-peptide coding re-

gion of the *lacZ* gene inserted into the *hobo* element in vector pHFL1. Plasmid pBR–*lacZ* contains the *lacZ* element from pHEB4 cloned into pBR322 with no *hobo*-element DNA associated with the insert. Cell line Hi5 is a *T. ni* embryonic cell line, and was purchased from Invitrogen (San Diego, CA). HzAM1 is a *H. zea* cell line originally developed by Dr. A. MacIntosh (University of Missouri, Columbia) and modified to a serum-free, non-adherent cell line and provided to us by Dr. B. Stiles (American Cyanamide, San Leandro, CA). Maintenance of both cell lines were under standard conditions.

Insect Rearing and Embryo Harvesting

The *H. zea* colony was obtained from Dr. Johnnie Jenkins (Mississippi State University, Stoneville). Pupae were introduced to a fixed phase-light cycle (15 hr light, 9 hr dark). On emergence of adults, matings were allowed to proceed for a maximum of 6 hr at a ratio of 3:1 (females/males) in mating cages lined with muslin cloth. After 6 hr, the cloth was removed, rinsed in 0.17% sodium hypochlorite for 2 min to remove adhering eggs. The eggs were then rinsed in 5000 volumes ddH_2O, unfertilized eggs removed based on buoyancy, and fertilized eggs transferred to cheese cloth to dry. Mating of *H. zea* adults was performed exactly as described by O.A. Johnson, K.J. Hughes, and J.D. DeVault (unpubl.). On identification of positive adults, individual moths were transferred to mating chambers with a single moth of the opposite sex. Mating chambers were lined with wax paper, contained cotton soaked in 10% sucrose, and were placed in an isolated incubation chamber on a 15-hr light, 9-hr dark cycle. Mating adults were transferred to fresh cages after 24 hr for three successive days. Eggs were collected each day and placed on artificial diet (Southland Products Inc., Lake Village, AR) to hatch.

Electroporation Conditions

One hundred individually treated embryos were placed into a specially designed micro-electroporation cuvette with a 2-mm gap (Leopold et al. 1996). A total of 40 μl of an aqueous solution of DNA (1μg/μl) was added to the cuvette and embryos were subjected to two pulses of 100 V, with a resistance of 13 Ω and capacitance of 600 μF. The cuvette was cooled on ice for 5 min to retard membrane pore resealing and increase DNA uptake. The electroporation device used was a BTX Electro Cell Manipulator (BTX, San Diego, CA).

hobo Excision Assay

The assay (Fig. 1) is a derivation of that described by Atkinson et al. (1993). Forty micrograms of plasmid DNA (pHEB4 or pBR–*lacZ*) was introduced into early blastoderm embryos of the corn earworm using electroporation, or into cell lines with lipofectamine reagent (GIBCO/BRL, Grand Island, NY). Excision of the *hobo* transposon was analyzed by isolating plasmid DNA from manipulated embryos and cell cultures, and transforming *lacZ*$^-$ *E. coli* strain JM109. Plasmid DNA was isolated by the method as described by Shahjahan et al (1995). Initial positive excision events were identified by the appearance of white colonies on MaConkey agar (GIBCO/BRL) containing tetracycline (30 μg/ml) following bacterial transformation. Successful excision was verified by examining digested plasmids for the absence of the *hobo–lacZ* insert using gel mobility analyses. Those plasmids lacking the appropriate insert were considered positive for insert excision.

Construction of Transgenic *H. zea* Moths

Embryonic electroporation was performed as described for the excision assay. At 16 hr post-electroporation, manipulated eggs were subjected to a 4-hr 37°C heat shock. Following heat shock, eggs were placed at 28°C and larvae allowed to emerge. Individual larvae were placed into separate rearing containers and allowed to pupate. Following pupation, individual pupal casings were subjected to a standard DNA extraction protocol (Shajahan et al. 1995), and PCR analysis performed using primers specific for the bacterial *lacZ* gene. PCR conditions were 93°C for 1 min, 62°C for 1 min, 70°C for 2 min for 30 cycles in a Perkin Elmer Thermal Cycler 480. The *lacZ*-specific primers used were: forward, CGCTGGTGAAAGTAAAAGATG; reverse, GATAACTACGATACGGGAGGGC. The PCR products were analyzed by agarose gel electrophoresis. Samples yielding 600 bp were considered positive. Following identification of adults harboring the *hobo–lacZ* insertion, backcross matings to wild-type adults were performed. The stability of the insert was determined as described above for successive generations.

DNA Transfer and Hybridization

DNA was transferred to Nytran nylon membrane exactly as described for the Turboblotter system (Schleicher & Schuell, Keene, NH). Hybridization of genomic and PCR-amplified DNA was performed exactly as described by Sambrook et al. (1989). The primer used to amplify to inserted *hobo*-end was CAGAGAACT(C/G)CA. Because both termini of the *hobo* element are identical, a single primer was used. All other molecular biological and microbiological techniques (including bacterial transformations and restriction digests) were exactly as described in Sambrook et al. (1989).

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