Bulletin of Entomological Research (2004) **94**, 95–110 DOI: 10.1079/BER2003290

REVIEW ARTICLE

Recent advances in transgenic arthropod technology

M.G. Kramer*

US Environmental Protection Agency, Office of Science Coordination and Policy, 1200 Pennsylvania Avenue NW, Mail Code 7201M, Washington, DC 20460, USA

Abstract

The ability to insert foreign genes into arthropod genomes has led to a diverse set of potential applications for transgenic arthropods, many of which are designed to advance public health or improve agricultural production. New techniques for expressing foreign genes in arthropods have now been successfully used in at least 18 different genera. However, advances in field biology are lagging far behind those in the laboratory, and considerable work is needed before deployment in nature can be a reality. A mechanism to drive the gene of interest though a natural population must be developed and thoroughly evaluated before any field release, but progress in this area has been limited. Likewise, serious consideration of potential risks associated with deployment in nature has been lacking. This review gives an overview of the most promising techniques for expressing foreign genes in arthropods, considers the potential risks associated with their deployment, and highlights the areas of research that are most urgently needed for the field to advance out of the laboratory and into practice.

Introduction

Insect pests continue to plague humans in spite of extensive efforts to chemically control them. Their detrimental impacts on agricultural production and their prominent role in transmission of many deadly diseases ensure that attempts to manage such pest populations will continue. The natural evolution of pesticide resistance and the inherent dangers to the environment and human health involved in large-scale application of pesticides have focused considerable effort on development of alternatives to chemical control. Scientists have sought to control insect pest populations through genetic manipulation since at least the 1930s (Lindquist, 1955). Recent advances in insect molecular biology have significantly broadened the available techniques for genetic manipulation of arthropod populations, the variety of traits and species that are of interest, and the potential applications of such technology. This diversity significantly complicates the consideration of potential risks presented by transgenic arthropods as a group. Thus each environmental release will present a unique set of risks and benefits that must be evaluated on a case-by-case basis. Another consequence of this diversity is an expansion of research areas that must be strengthened before widespread adoption of this technology for public health, agricultural, and other uses can be reasonably contemplated. This review will highlight the diversity of recent advances in transgenic arthropod technology, consider how the potential risks overlay upon this diversity, and suggest areas of research that are most urgently needed if this technology is to move from the laboratory to the field.

*Fax: +1 202 564 8502
E-mail: kramer.melissa@epa.gov
The views presented here are solely those of the author and do not necessarily represent the views of the US EPA or the US government.

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press

The goals of arthropod genetic transformation

Releases of genetically-modified arthropods as part of population suppression programmes has occurred for decades with relatively little controversy (see Braig & Yan, 2002 for review). In the last 20 years the potential utility of such programmes and the possible applications of genetically-modified arthropods have expanded considerably. Largely responsible for this expansion is the technique of genetic transformation, the incorporation of foreign DNA into a host genome so that it can be transmitted to later generations in a stable, predictable manner. This technique has expanded scientific possibilities but also raised serious scientific questions about the deployment of genetically modified arthropods.

The first genetically transformed insects were produced 20 years ago when wild-type eye colour was restored in a mutant strain of the vinegar fly *Drosophila melanogaster* Sturtevant (Diptera: Drosophilidae) by insertion of a DNA fragment containing a wild-type copy of the defective eye-colour gene (Rubin & Spradling, 1982). Transformation of *D. melanogaster* has since become a standard technique in many laboratories that is a powerful tool for basic biology investigations. Sophisticated methods that rely on transformation-based techniques have now been developed in *D. melanogaster* to identify cloned genes and determine their function, e.g. gene tagging and enhancer trapping. Much of the progress in *Drosophila* transgenesis has indeed been motivated by such applications.

The ability to transform non-drosophilid arthropods can be expected to open the door to future advances in basic biology with other species. Progress with other insects lags far behind *Drosophila* because significant advances did not occur until after the Mediterranean fruit fly (medfly), *Ceratitis capitata* Weidemann (Diptera: Tephritidae) was transformed in 1995 (Loukeris *et al*.). Foreign genes have since been expressed in at least 18 different arthropod genera (table 1), and progress is now proceeding rapidly on several fronts.

Most species are initially transformed with a marker gene such as green fluorescent protein (GFP). Once the technical hurdles associated with transformation have been overcome, the search begins for genes that will express a desired trait in the transformed arthropod. These traits and the overall goals of transformation are as varied as the insects expressing them. A notable characteristic of transgenic arthropod technology is the extent to which research and anticipated applications are occurring in the public domain for public benefit.

Although eradication is a common insect pest control strategy, it is not realistic for many insect pests due to biological constraints, and it may even be undesirable if it merely produced an empty ecological niche that might be readily filled by new immigrants (Curtis, 1994). The goal of transforming an arthropod species is often to replace a natural population with individuals genetically modified such that they are no longer pests. Both agricultural pests and disease vectors are likely targets of such programmes.

Beneficial species transformed with a variety of traits could be deployed as more effective biocontrol agents. For example, the first field trials of transgenic arthropods have already been conducted with a transgenic predatory mite, *Metaseiulus occidentalis* Nesbitt (Acari: Phytoseiidae; Presnail *et al*., 1997). It is a predator of spider mites including *Tetranychus urticae* Koch (Acari: Tetranychidae), a pest in deciduous orchards and vineyards in the western United States. If natural populations of predatory mites could be replaced with transgenic conspecifics that have been modified for pesticide resistance, pesticide applications could be reduced as they would affect the agricultural pests while leaving their natural predators unharmed.

Scientists would like to suppress the ability of insects to serve as vectors of human and animal pathogens. Vector-borne diseases continue to plague human populations in spite of extensive control programmes. Population growth and increased urbanization have occurred at the same time that many public health efforts to control transmission have declined (Beaty, 2000). Still no effective vaccines exist against major diseases such as malaria, Chagas disease and sleeping sickness. Not surprisingly, considerable effort has been directed towards transformation of vectors of these important human illnesses with the goal of creating insects that are either refractory to parasites (i.e. do not support their development) or are unable to transmit the parasite to their vertebrate hosts.

Another potential modification of haematophagous insects is the insertion of transgenes such that the insects could serve as vaccine delivery vehicles (Crampton *et al*., 1999). A blood-feeding insect could be transformed to express an antigen in its saliva that would be delivered to the vertebrate host during each blood meal. Experiments have already demonstrated the feasibility of producing a recombinant insect with tissue-specific expression, and mosquito cells have been transformed with an appropriate antigen gene (Coates *et al*., 1999; Crampton *et al*., 1999).

While most applications involve modification of pest species, some transgenic insects are desired for commercial production of materials, analogous to the creation of genetically-engineered agricultural crops. Silkworms (*Bombyx mori* Linnaeus; Lepidoptera: Bombycidae); and honeybees (*Apis* spp.; Hymenoptera: Apidae) have been subjects of artificial breeding programmes for decades. Genetic transformation offers the possibility of a more rapid process and the introduction of a wider variety of traits, e.g. the production of new biomaterials by the modification of silk protein (Mori & Tsukada, 2000) or the production of pharmaceuticals and biomolecules such as human growth hormone (Kadonookuda *et al*., 1995; Ho *et al*., 1998; Yang *et al*., 2002).

The goals of genetic transformation of arthropod species will be intimately connected to: (i) the techniques used for transformation, as each involves a unique set of risks; (ii) risk assessment, as the goals highlight the potential benefits; and (iii) public perception and acceptance of this technology, as society is more likely to accept any risks or costs of deployment if it finds the goals worthy enough.

The techniques of arthropod genetic transformation

Early methods for genetic modification of arthropods relied on very crude manipulations such as irradiation that required little to no knowledge of the underlying genetic system or how exactly it was altered. Genetic transformation offers far greater precision by allowing the insertion of specific, desired genes. It is this ability to insert essentially any gene for expression in insects that has created concern over transgenic arthropod releases. However, unlike with plants, in which the transformation technique has become

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press

secondary to the trait conferred in performing a risk assessment, the transformation technique is central to the evaluation of potential risks associated with release of transgenic arthropods.

Transposable elements

Transformation in *D. melanogaster* relies on so-called *P* elements, mobile pieces of DNA that do not remain fixed at one genomic location, but rather have the ability to move from one site on a chromosome to another (Liao *et al*., 2000). *P* elements are just one class of such mobile DNA pieces that are collectively called transposable elements. They can increase their copy number as they move around among chromosomes within individual organisms. They are vertically transmitted to progeny and can likely move between individuals of different species in a process known as horizontal gene transfer (Kidwell, 1992). The behaviour of transposable elements is central to their success as transformation vectors but is also the source of many scientific questions that must be answered before deployment of an insect population transformed with a transposable element (see below).

Transposable elements are divided into two classes. Class I elements use an RNA intermediate while Class II elements transpose directly from DNA to DNA (Finnegan, 1989). Scientists have manipulated a subset of Class II transposable elements to introduce foreign DNA into whole insects. These elements have short terminal sequences in an inverted repeat orientation and a transcriptional unit that encodes for a protein (a transposase) that is needed to facilitate the precise excision of the transposable element and its insertion into another region of the genome (Rubin & Spradling, 1982; Spradling & Rubin, 1982). Importantly, the transposase can function *in trans*, meaning that it can recognize the inverted terminal repeats of a transposable element other than the one in which it is located to catalyse its transposition. Understanding the activity of endogenous transposases is also central to predicting the behaviour of introduced transposable elements, but, as with the activity of transposable elements, this aspect is insufficiently understood.

The use of *P* elements to transform insects begins by microinjecting embryos with two circular DNA plasmids, one called the vector and the other called the helper. The vector plasmid contains: (i) a transposable element; (ii) the exogenous DNA that is to be incorporated into the host genome, placed under the control of a promoter than can provide tissue and/or temporal expression specificity; and (iii) a marker gene that can be used to identify the individuals that have actually inserted the exogenous DNA. The vector lacks a transposase so that the construct will be unable to mobilize itself once inserted, and the transposase is instead provided by the helper plasmid. Although able to produce transposase, the helper is unable to incorporate itself into the genome because it lacks the inverted terminal repeat region of the transposable element. Thus the helper plasmid catalyses the excision of the exogenous DNA plus marker gene from the vector plasmid and their insertion into the host genome (Rubin & Spradling, 1982). Injected individuals are then outcrossed, and the progeny are examined for expression of the marker phenotype. Segregation of this phenotype in Mendelian ratios suggests chromosomal insertion of the transgene (Rubin & Spradling, 1982). The structure of the transposable element and its incorporation into the genome of transformed insects is verified by Southern blot analyses and/or PCR amplification.

In non-drosophilid insects, transformation follows the same paradigm as for *P* elements but using different transposable elements. After well over a decade of failed attempts, *P* elements were determined not to work outside the genus *Drosophila*, likely due to either endogenous repression or missing host-encoded factors (Atkinson & James, 2002). Four different families of Class II transposable elements unrelated to *P* elements have now been used to transform species other than *D. melanogaster*: *Tc1* elements, *mariner* elements, *hAT* elements, and *piggyBac* elements. Each family is united by sequence similarity and general structural characteristics.

Minos is the only member of the *Tc1* family of transposable elements found in insects. It was isolated from *Drosophila hydei* Sturtevant (Diptera: Drosophilidae), and was used in the first non-drosophilid transformation with a transposable element: the medfly, *C. capitata* (Loukeris *et al*., 1995). To date, only one additional insect species has been transformed with *Minos*: the mosquito *Anopheles stephensi* Liston (Diptera: Culicidae; Catteruccia *et al*., 2000).

Mariner elements consist of several subfamilies that are widely distributed across at least six different insect orders and are found in at least ten different insect species (Robertson, 1993). This broad host range and the discovery of thousands of copies from multiple subfamilies within single genomes suggest that this element has undergone rampant horizontal gene transfer (Lohe *et al*., 1995). *Mariner* was originally isolated in *Drosophila mauritiana* (Diptera: Drosophilidae; Jacobson *et al*., 1986) and has since been used to successfully transform three insect species in addition to *D. melanogaster* (Lidholm *et al*., 1993; table 1). *Mariner* has also been found to be active in at least two additional insect species using an interplasmid transposition assay in the cells of developing embryos: the Australian sheep blowfly *Lucilia cuprina* Wiedemann (Diptera: Calliphoridae) and the Queensland fruit fly *Bactrocera tryoni* Froggatt (Diptera: Tephritidae; Coates *et al*., 1997). Such assays indicate the potential of transposable elements to serve as vectors in the test species (i.e. transpose while carrying non-element DNA).

hAT elements, like *mariner* elements, have an extremely broad host range having been found in plant, animal and fungus species (Rubin *et al*., 2001). Two different insect *hAT* elements have been used as transformation vectors: *Hermes* and *hobo*. *Hermes* was isolated from the housefly *M. domestica* and was found to functionally transpose in *D. melanogaster* at frequencies comparable to *P* element transformation (O'Brochta *et al*., 1996) as well as in several other insect species (table 1). *Hobo* was isolated from *D. melanogaster* and found to effectively transform both this species (Blackman *et al*., 1989) and *D. virilis* (Lozovskaya *et al*., 1996). Interplasmid transposition assays have shown *hobo* mobility in non-drosophilid insects including the housefly *M. domestica*, the Queensland fruit fly *B. tryoni* (O'Brochta *et al*., 1994), and the corn earworm *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae; Pinkerton *et al*., 1996).

PiggyBac elements were first isolated from the cabbage looper moth *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae). It has been a tremendously successful transposable element having been used to successfully transform a dozen non-drosophilid species (table 1).

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press

Table 1. List of non-drosophilid insects that have been engineered to express foreign genes *in vivo* including the introduced trait and method of introduction.

Species	Expressed trait	Method	Reference
Ceratitis capitata (Wiedemann) (Diptera: Tephritidae)	Wild-type eye colour	*Minos* transposable element	Loukeris *et al*., 1995
	Wild-type eye colour	*PiggyBac* transposable element	Handler *et al*., 1998
	Wild-type eye colour	*Hermes* transposable element	Michel *et al*., 2001
Bombyx mori (Linnaeus) (Lepidoptera: Bombycidae)	Biomolecule production (e.g. carp growth hormone, human growth hormone, human calcitonin)	Baculovirus infection	Kadonookuda *et al*., 1995; Ho *et al*., 1998; Yang *et al*., 2002
	Green fluorescent protein	*PiggyBac* transposable element	Toshiki *et al*., 2000
	Green fluorescent protein	Homologous recombination with a baculovirus (AcNPV)	Yamao *et al*., 2002
Cardiochiles diaphaniae Marsh (Hymenoptera: Braconidae)	Resistance to parathion	Maternal microinjection of plasmid DNA	Presnail & Hoy, 1996
Aedes aegypti (Linnaeus) (Diptera: Culicidae)	Inhibition of DEN-2 virus replication in salivary glands	dsSIN transducing virus	Olson *et al*., 1996
	Resistance to yellow fever virus	dsSIN transducing virus	Higgs *et al*., 1998
	Wild-type eye colour	*Mariner* transposable element	Coates *et al*., 1998, 2000
	Wild-type eye colour	*Hermes* transposable element	Jasinskiene *et al*., 1998
	Luciferase expression	Pantropic retroviral vector	Jordan *et al*., 1998
	Luciferase expression inhibition	dsSIN transducing virus	Johnson *et al*., 1999
	Resistance to salivary gland sporozoite infections	dsSIN transducing virus	de Lara Capurro *et al*., 2000
	Defensin A, an insect microbicide	*Hermes* transposable element	Kokoza *et al*., 2000
	Green fluorescent protein	dsSIN transducing virus administered orally	Olson *et al*., 2000
	Green fluorescent protein	*Hermes* transposable element	Pinkerton *et al*., 2000
	Green fluorescent protein	Transducing densovirus (*Ae*DNV)	Afanasiev *et al*., 1999; Ward *et al*., 2001
Aedes triseriatus (Say) (Diptera: Culicidae)	Resistance to LAC virus infection	dsSIN transducing virus	Powers *et al*., 1996
Anopheles stephensi Liston (Diptera: Culicidae)	Green fluorescent protein	*Minos* transposable element	Catteruccia *et al*., 2000
	Red fluorescent protein	*PiggyBac* transposable element	Nolan *et al*., 2002
	Antiparasitic genes	*PiggyBac* transposable element	Ito *et al*., 2002
Anopheles gambiae Giles (Diptera: Culicidae)	Green fluorescent protein	Transducing densovirus (*Ae*DNV)	Afanasiev *et al*., 1999
	Green fluorescent protein	*PiggyBac* transposable element	Grossman *et al*., 2001
Anopheles albimanus Wiedemann (Diptera: Culicidae)	Green fluorescent protein	*PiggyBac* transposable element	Perera *et al*., 2002
Culex quinquefasciatus Say (Diptera: Culicidae)	Green fluorescent protein	*Hermes* transposable element	Allen *et al*., 2001
Rhodnius prolixus Stål (Hemiptera: Reduviidae)	Expression of cecropin A, a trypanocidal protein	Paratransgenesis using *R. rhodnii*	Durvasula *et al*., 1997
	Expression of a functional antibody fragment	Paratransgenesis using *R. rhodnii*	Durvasula *et al*., 1999a
Manduca sexta (Johannsen) (Lepidoptera: Sphingidae)	Infection in antennal cells	Pantropic retroviral vector	Franco *et al*., 1998
Glossina spp. (Diptera: Glossinidae)	Green fluorescent protein	Paratransgenesis using S-symbionts	Cheng & Aksoy, 1999
Tribolium castaneum Herbst (Coleoptera: Tenebrionidae)	Green fluorescent protein	*Hermes* and *Piggybac* transposable elements	Berghammer *et al*., 1999
Bactrocera dorsalis (Hendel) (Diptera: Tephritidae)	Wild-type eye colour	*PiggyBac* transposable element	Handler & McCombs, 2000
Stomoxys calcitrans Linnaeus (Diptera: Muscidae)	Green fluorescent protein	*Hermes* transposable element	O'Brochta *et al*., 2000
Pectinophora gossypiella Saunders (Lepidoptera: Gelechiidae)	Green fluorescent protein	*PiggyBac* transposable element	Peloquin *et al*., 2000
Apis mellifera Linnaeus (Hymenoptera: Apidae)	Green fluorescent protein	Sperm-mediated transformation	Robinson *et al*., 2000

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press

Species	Expressed trait	Method	Reference
Musca domestica Linnaeus (Diptera: Muscidae)	None; PCR screening Green fluorescent protein	*Mariner* transposable element *PiggyBac* transposable element	Yoshiyama *et al*., 2000 Hediger *et al*., 2001
Anastrepha suspensa Loew (Diptera: Tephritidae)	Green fluorescent protein	*PiggyBac* transposable element	Handler & Harrell, 2001
Lucilia cuprina (Wiedemann) (Diptera: Calliphoridae)	Green fluorescent protein	*PiggyBac* transposable element	Heinrich *et al*., 2002
Athalia rosae Linnaeus (Hymenoptera: Tenthredinidae)	Green fluorescent protein	*PiggyBac* transposable element	Sumitani *et al*., 2003

An analysis of the *Drosophila* genome revealed 85 known and eight new families of transposable elements (Kaminker *et al*., 2002). Assessment of their activity and the search for additional transposable elements in other arthropods is likely lead to additional transformation vectors.

Sperm-mediated transformation

In spite of the dramatic progress that has been made with genetic transformation systems based on transposable elements in non-drosophilid insects, it remains a labour-intensive process that will likely never be a viable method of transformation for some species. Limitations on its usefulness are imposed by characteristics such as long generation times, low reproductive rates, or egg properties that prevent DNA introduction. The need to microinject DNA into developing embryos has been a major obstacle for transformation of some species. For example, embryos of the honeybee *Apis mellifera* Linneaus (Hymenoptera: Apidae) that have been manipulated during microinjection will be destroyed by nurse worker bees if reintroduced into the hive, and successful protocols for laboratory rearing of embryos are unknown (Kimura, 2001). DNA has been successfully introduced into individual honeybees by instrumentally inseminating virgin queens with a mixture of linearized DNA and semen (Robinson *et al*., 2000). Although there is no evidence that the DNA was incorporated into the genome, it was stably inherited over three generations, likely as extrachromosomal arrays. Using this technique in combination with transposable elements may eventually allow stable germline integration of exogenous DNA, but progress has been limited.

Viral vectors

Viral systems offer additional promising techniques for expression of foreign genes *in vivo*. Most viral systems lack the stable inheritance of transposable elements that would be needed for deployment in nature. Nevertheless, these techniques are ideal for understanding the *in vivo* function of particular genes and identifying those that would be good candidates for transformation.

The Sindbis alphavirus

Alphaviruses are normally infectious to vertebrates through mosquito vectors. The prototype alphavirus, Sindbis (SIN), consists of a single strand of RNA (49S) that is considered by convention to be positive-sense because it serves as the mRNA template for translation (Strauss *et al*., 1984). Polymerase components are translated from the 5′ two-thirds of this strand, and these are used to synthesize a full-length minus-sense strand. This minus-sense strand in turn serves as a template for synthesis of both full-length and subgenomic strands including a 26S subgenomic mRNA that is translated to produce a polyprotein. Post-translational cleavage of this polyprotein produces structural proteins that are necessary for production of a mature virion (Strauss *et al*., 1984).

Viral transducing systems have been created from the SIN alphavirus that allow long-term and stable cytoplasmic expression of foreign DNA in mosquitoes. The double subgenomic SIN (dsSIN) expression system uses a full-length SIN cDNA that has an additional internal promoter (Hahn *et al*., 1992). Foreign DNA can be inserted downstream of this promoter so that cells that are infected with dsSIN RNA transcribe this foreign DNA in addition to the full-length mRNA and subgenomic mRNA that encodes the viral proteins. Thus infectious, recombinant virions are produced that can be used to transmit the foreign gene to other cells and achieve a high level of expression that is long-term due to the persistent, non-cytopathic nature of the infection in mosquitoes.

Alphaviruses are infectious to humans and other vertebrates and are thus broadly recognized as inappropriate for use in an insect intended for environmental release. Nevertheless, the dsSIN expression system is a powerful method for identifying genes most appropriate for use with transposable element-based transformation systems described above. Some of the most promising results in the field of insect transgenesis have occurred with this system because it has demonstrated that insects can be manipulated to express phenotypes that would be effective at achieving public health goals. For example, the dsSIN system has been used to express an anti-circumsporozoite monoclonal antibody in *Aedes aegypti* (Linnaeus) (Diptera: Culicidae) that is effective in substantially reducing the level of *Plasmodium gallinaceum* Brumpt (Haemosporida: Plasmodiidae) sporozoite infection in salivary glands (de Lara Capurro *et al*., 2000). Powers *et al*. (1996) showed that expression of LaCrosse virus sequences using the dsSIN system would inhibit LaCrosse virus infection in *Aedes triseriatus* Say (Diptera: Culicidae) based on the fact that in mosquitoes superinfection with alphaviruses generally does not occur, that is, a cell infected by one alphavirus inhibits infection by a second, homologous virus (Karpf *et al*., 1997). Similarly in *A. aegypti*, expressing part of a Dengue virus genome inhibited virus transmission (Olson *et al*., 1996), and resistance to yellow fever virus was accomplished by using dsSIN viruses engineered with antisense RNA complementary to yellow fever virus sequences (Higgs *et al*., 1998). Recent advances in this technique have allowed expression of a transgene in the

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press

mosquito midgut after bloodmeal delivery, followed by expression in other target tissues the virus naturally infects (Pierro *et al*., 2003). The number of candidate genes potentially able to render a mosquito refractory to disease transmission that could be tested relatively easily with the dsSIN expression system is likely to increase with publication of the *Plasmodium* and *Anopheles* genomes (Gardner *et al*., 2002; Holt *et al*., 2002) and as the number of quantitative trait loci identified for refractoriness increases (Severson *et al*., 1995; Gorman *et al*., 1997; Zheng *et al*., 1997).

Densoviruses

Densoviruses, small DNA viruses that are infectious to a variety of insects and crustaceans, have also been developed into gene expression vectors in mosquitoes. They too produce only transient expression because site-specific integration into the host genome does not appear to occur, although the introduction of a site resembling the densovirus replication origin into the host genome could perhaps enable integration (Afanasiev & Carlson, 2000). They have the advantage of relatively narrow host specificity in spite of being widespread in nature, and their structure makes them relatively stable in the environment. In addition, they can infect mosquito larvae grown in cell culture medium containing transducing viral particles, so microinjection is unnecessary (Barreau *et al*., 1994).

To create a transducing genome, the viral genes in an infectious clone are replaced by the gene of interest. Only the terminal inverted repeats that enable replication and packaging of the genome must be retained, but a helper construct is needed to provide the missing viral genes. The transducing genome is then delivered to cells by infection (Afanasiev *et al*., 1994). A major limitation of this cotransfection method is that in addition to transducing particles, it also generates wild-type virus lacking the gene of interest due to recombination between the transducing genome and helper constructs. An alternative is the use of the dsSIN expression system as a vehicle to express both the viral function proteins and the densovirus genome (Allen-Miura *et al*., 1999).

Densoviruses from *A. aegypti* and *Junonia coenia* Hübner (Lepidoptera: Nymphalidae), *Ae*DNV and *Jc*DNV respectively, have been used to create transducing vectors that express a reporter gene (lacZ) in insect cells (Giraud *et al*., 1992; Afanasiev *et al*., 1994). Successful transduction of *A. aegypti* and *Anopheles gambiae* Giles (Diptera: Culicidae) larvae was accomplished with a transducing particle containing a green fluorescent protein (GFP) using a helper plasmid to supply viral function proteins (Afanasiev *et al*., 1999; Ward *et al*., 2001). Although densoviruses are potentially very valuable for arthropod transgenesis, a considerable amount of further work is needed to develop the technique into a system as useful as the dsSIN system.

Baculoviruses

The *Autographa californica* Speyer (Lepidoptera: Noctuidae) nucleopolyhedrovirus (AcNPV) has also been developed into a expression vector for foreign genes that can be maternally transmitted in the silkworm, *B. mori* for several generations (Mori *et al*., 1995). Homologous recombination between the baculovirus and the host genome allows specific alterations of gene sequences rather than the uncontrolled insertion of foreign DNA that occurs when using transposable elements (Yamao *et al*., 2002). The baculovirus expression system has potential to enable modification of silk proteins and the use of silkworms for production of other useful proteins such as human growth hormone, carp growth hormone and human calcitonin (Kadonookuda *et al*., 1995; Ho *et al*., 1998; Mori & Tsukada, 2000; Yang *et al*., 2002). The system has not demonstrated potential usefulness other than with *B. mori*, although much of the research in this area is undoubtedly occurring outside of the public domain because of its probable applications.

Paratransgenesis

In species that harbour an obligate symbiotic microorganism, foreign genes may be expressed by actually transforming the endosymbiont rather than the insect itself. This technique is called paratransgenesis and is the most promising avenue of research in tsetse flies and kissing bugs. Although not strictly transgenic, such insects raise many of the same potential risk issues and are considered here for that reason.

Tsetse flies

Tsetse flies (Diptera: Glossinidae) are vectors of trypanosomes, the causative agent of sleeping sickness (trypanosomiasis) and other diseases. Germline transformation systems that rely on microinjection are unfeasible because tsetse flies are viviparous, holding their eggs internally until after hatching (Aksoy *et al*., 2001). Tsetse flies harbour three different bacterial endosymbionts that are transmitted to larvae *in utero* (Dale & Welburn, 2001). The primary symbionts, *Wigglesworthia* spp. (Enterobacterialis: Enterobacteriaceae) reside in specialized gut cells. The secondary symbionts, *Wolbachia* spp. (Rickettsiales: Rickettsiaceae) and *Sodalis* spp. (Enterobacterialis: Enterobacteriaceae), are found primarily in ovarian tissue and midgut tissue respectively. These endosymbionts are found in close proximity to trypanosomes which differentiate in the midgut and then travel to the salivary glands for maturation before transmission to a new host.

Genetically engineering one of the endosymbionts to express antitrypanosomal agents in the gut of tsetse flies could prevent transmission of sleeping sickness (Aksoy, 2000). Recombinant *Sodalis* have been successfully cultured and engineered to express the marker trait GFP. When introduced into fertile females, F_1 and F_2 progeny also carry endosymbionts expressing GFP, suggesting that such recombinant progeny could be used to transmit foreign DNA that had the desired properties (Cheng & Aksoy, 1999). Identification of agents that could be used to reduce the vector competence of tsetse flies through expression in *Sodalis* is advancing, including the isolation of three antimicrobial genes expressed in tsetse fat body tissue that appear to modulate infection in the insect (Hao *et al*., 2001). Flies whose first meal consisted of infectious trypanosomes along with recombinant *Sodalis* expressing the anti-microbial peptide attacin had their infection rate reduced from 80% to zero, while adult flies experienced a lower but still significant reduction (Aksoy, 2003).

Kissing bugs

The kissing bug, *Rhodnius prolixus* Stål (Hemiptera: Reduviidae), is a vector of *Trypanosoma cruzi* Chagas

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press

(Kinetoplastida: Trypanosomatidae), the causative agent of Chagas disease. An obligate bacterial symbiont of *R. prolixus*, *Rhodococcus rhodnii* Goodfellow & Alderson (Corynebacterineae: Nocardiaceae), lives in the gut lumen in close proximity to *T. cruzi*, making kissing bugs ideal candidates for paratransgenesis. *Rhodococcus rhodnii* has been transformed with a marker gene (Beard *et al*., 1992) and with a gene for cecropin A, a trypanocidal peptide found in insect hemolymph (Durvasula *et al*., 1997). When *R. prolixus* was infected with transgenic *R. rhodnii*, cecropin A was stably expressed and effectively killed *T. cruzi* without otherwise damaging the kissing bugs. Other experiments showed the feasibility of expressing active mammalian single-chain antibodies in *R. prolixus* using paratransgenesis, although none had actual *T. cruzi* transmission-blocking ability (Durvasula *et al*., 1999a). Research with paratransgenic kissing bugs has advanced beyond that of any other species discussed here because it has progressed from the laboratory to evaluation of strategies for actual deployment in nature (discussed below).

Marker systems

Arthropod transformation using the methods described above would be seriously hampered without an appropriate marker system in each species to identify which insects were actually expressing the exogenous DNA (Kidwell & Wattam, 1998). Early markers relied on the existence of a mutant strain and identification of a corresponding gene that could restore the wild-type phenotype (Rubin & Spradling, 1982; Loukeris *et al*., 1995; Cornel *et al*., 1997; Jasinskiene *et al*., 1998). Although useful when available, such marker systems severely hinder the advance of insect transformation in a broad range of species because they require isolation of appropriate mutants and cloning of the corresponding genes.

The first markers that enabled efficient selection of transformants were chemical or drug resistance genes. The organophosphate-degrading enzyme conferred resistance to the pesticides paroxon and parathion when expressed in *D. melanogaster* (Benedict *et al*., 1994, 1995) and the endoparasitoid wasp *Cardiochiles diaphaniae* Marsh (Hymenoptera: Braconidae; Presnail & Hoy, 1996). Chemical resistance genes proved to be particularly difficult to use in practice because generalized protocols were unavailable and the repeated selection pressure usually produced non-vector-related resistant insects due to the natural evolution of this trait. In addition, chemical resistance genes in insects that are intended for release create unnecessary risks because of the danger that they could be transferred to a non-target organism. Such considerations must arise for whatever marker is used because of the chance that it could be transferred into an organisms in which it was not intended to be. Pesticide resistance has been used in arthropod transformation when the goal has been to actually express this trait in beneficial species, e.g. predatory mites (Hoy, 2000).

The most important dominant marker system that has been developed has been the green fluorescent protein (GFP) marker and its modified forms. This protein was isolated from the jellyfish *Aequorea victoria* Murbach & Shearer (Conica: Aequoreidae) and found to be active across the animal and plant kingdoms, including in arthropods (Tsien, 1998; Pinkerton *et al*., 2000). It produces green fluorescence in the tissues where it is expressed and can be used to identify transformants even in wild-type strains (Berghammer *et al*., 1999). The GFP gene may be linked with different promoters that allow expression in appropriate tissues for easy detection in different insects. This has been the marker of choice for nearly every recent insect transformation.

Promoters

By creating constructs with different promoters, expression of transgenes can be limited spatially and/or temporally. Many transformation experiments have used the promoter of the heat shock protein hsp70 from *D. melanogaster* to allow temporal control of gene expression with temperature change. Promoters that allow tissue specificity enable targeting gene expression to the most appropriate regions for controlling parasite viability or other purposes. Two genes, *Maltase-like I* and *Apyrase,* are expressed in female mosquito salivary glands, the site of viral replication and the last location of parasites and viruses before they leave the mosquito to infect a vertebrate host. When the promoters for these genes drive expression of a reporter gene in *A. aegypti*, the gene has the expected sex-, stage- and tissue-specificity (Coates *et al*., 1999). The *vitellogenin (Vg)* gene is expressed in the fat body of female mosquitoes after a blood meal, the time when parasite transmission from vertebrate hosts to mosquitoes occurs. In mosquitoes transformed with a construct using the *Vg* promoter to drive expression of an agent with strong anti-*Plasmodium* activity, a bloodmeal triggered expression of antipathogen factors in the mosquito fat body (Kokoza *et al*., 2000). The carboxypeptidase promoters of *A. gambiae* can also be used to drive blood-inducible gene expression in the gut of transgenic *A. aegypti,* a significant achievement considering that all parasites must travel through the mosquito gut for transmission to their vertebrate hosts (Moreira *et al*., 2000).

Drive mechanisms

For most applications of transgenic arthropods, successfully transforming the species is only the first technological hurdle. Equally, if not more, difficult is the development of a strategy for replacement of natural populations with recombinant individuals when desired. Strategies for fixing beneficial genes in insect pest populations have been discussed for at least 35 years (Curtis, 1968), but noteworthy advances have been few and far between. The simple mass release of insects with a novel trait but equal fitness to individuals in wild populations would be inadequate for fixing the new gene in the population. A 'drive mechanism' is needed to actively spread the trait through the population by either directly or indirectly eliminating individuals lacking the trait (Ribeiro & Kidwell, 1994).

Much effort in the field has been devoted to developing transgenic mosquitoes, but the requirements for replacing a natural mosquito population with one unable to transmit a malarial parasite are particularly daunting. Fixation of the gene must be ensured because more than 99% of the mosquitoes in an area must be refractory for a reduction in malaria transmission to actually occur (Braig & Yan, 2002). Releases that fall short of this goal are likely to make no impact and could even increase the rate.

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press

Ideally, the transgenic strain would have greater fitness than the wild population it was intended to replace, but this is rarely, if ever, naturally the case. Experiments with transgenic *A. stephensi* found that either the transgene itself or a characteristic of the insertion process was responsible for reducing fitness in three out of four mosquito lines (Catteruccia *et al*., 2003). The introduction of novel pesticide resistance genes into transgenic strains could cause them to have greater fitness than wild-type populations with pesticide applications that would serve as a powerful selective force favouring the introduced insects. However, experiments with a parasitoid of the walnut aphid that had been artificially selected (not genetically modified) for resistance to a pesticide showed that both replacement and introgression were occurring over a several-year period and that the process of establishment was more complicated than initially imagined (Hoy, 2000).

Transposable elements used to introduce genes into individual insects may potentially be used for driving the genes through populations (Meister & Grigliatti, 1993; Carareto *et al*., 1997). Transposable elements replicate within individuals, giving them an advantage over any gene inherited in a Mendelian fashion. When an individual with a transposable element mates with one lacking the element, it may multiply and eventually spread to all of the chromosomes in the hybrid genome even if the transposable element carries a selective disadvantage (Brookfield, 1986). When all of the gametes of a hybrid carry transposable elements, they could theoretically be driven to fixation in the population even if they reduced fitness as long as the reduction were less than 50% (Kidwell & Ribeiro, 1992). Such a mechanism is believed to have driven the *P* element to fixation in wild populations of *D. melanogaster* within the last century (Kidwell, 1992), but little information is known about the population dynamics of transposable elements carrying cargo genes or whether they would stay linked as the transposable element spread (Enserink, 2000). A *P* element carrying a functional alcohol dehydrogenase (*Adh*) gene was found to spread through populations of *D. melanogaster* at rates comparable to unmodified *P* elements, and the *Adh* gene appeared to retain functional activity in a high proportion of cases (Meister & Grigliatti, 1993). Other evidence suggests a negative correlation between the maintenance of construct integrity and transposition rate (Carareto *et al*., 1997).

Natural mechanisms appear to limit the mobility of transposable elements. After *P* elements spread through a population, the activity of the transposase often decreases, possibly under selective pressure for reduction in the genome disruption that transpositions can cause (Brookfield, 1986; Kidwell & Ribeiro, 1992). Transposable elements are generally found in both active and inactive forms, the inactive forms being partially deleted elements that may serve as repressors of transposition (O'Hare & Rubin, 1983; Daniels *et al*., 1987). When two *P* element-containing individuals mate, repressors function in each individual to prevent transposition. Only when one individual lacks any element at all (intact or deleted), is the element able to transpose to a new individual (Kidwell & Ribeiro, 1992). Such phenomena should be well understood before transposable elements are utilized as a drive mechanism because a given transposable element may only be an effective agent for spreading a gene through a population a single time. If a gene were intentionally or accidentally introduced into the population using a transposable element, the introduction of additional genes using this element may not be possible. Such secondary introductions may be wanted if the introduced phenotype were not as predicted or desired.

Additional experiments are sorely needed to evaluate the population dynamics of transposable elements in a variety of systems. Experiments with the *hobo* transposable element have demonstrated that the experimental and theoretical work done with *P* elements may not be applicable to other transposable elements (Galindo *et al*., 1995; Ladevèze *et al*., 1998, 2001). Progress understanding the likely behaviour of transposable elements in non-drosophilids is likely to be seriously hampered if results are found to have limited applicability across families because almost all work in the field has concentrated on *P* elements. The critical parameter values needed to ensure efficient and reliable transgene spread are likely to vary considerably with insect species, transposable element, and characteristics of the cargo gene (Preston & Engels, 1989; Meister & Grigliatti, 1993).

The method for driving a gene into a natural population that is most often discussed for tsetse flies involves the use of one of their endosymbionts, *Wolbachia*. A large number of insects have been found to have *Wolbachia* infections, due in large part to the reproductive abnormalities that this bacterium causes. Infected individuals acquire a reproductive advantage over their conspecifics due to so-called cytoplasmic incompatibility. Generally, infected females can mate with all males, but uninfected females must mate with uninfected males to produce viable offspring. The reproductive advantage this provides infected females is enough to drive the infection through a population in a relatively short time (Collins & Paskewitz, 1995). If the *Wolbachia* symbiont itself were transformed with a transgene that could inhibit infection transmission, then that phenotype could be driven through the population with the endosymbiont. Currently, however, no *Wolbachia* transformation experiments have been reported and prospects for use of this technology are uncertain.

Theoretical evaluations of *Wolbachia* population dynamics indicate relatively stringent requirements for using this as a drive mechanism for transgenes (Turelli & Hoffmann, 1999). Population experiments were conducted using two strains of *M. occidentalis*: one with a natural *Wolbachia* infection and one that was cured of infection by heat treatment (Johanowicz & Hoy, 1999). With a 1:9 ratio respectively, the infection failed to sweep through the population over eight generations due to the fitness costs of infection. Ratios much greater than this might be able to compensate for the reduction in fitness, but they are considered to be economically unviable in most instances.

A method for the spread of transgenes through populations of kissing bugs uses their natural coprophagic behaviour (faeces consumption) that is responsible for transmission of *R. rhodnii* from generation to generation. An artificial faeces preparation (CRUZIGARD) that contains transformed endosymbionts has been produced (Durvasula *et al*., 1997). Simulated field trials have shown that CRUZIGARD is an effective means of spreading foreign genes (specifically an antibody fragment) through populations of kissing bugs (Durvasula *et al*., 1999b). Since newly emerged kissing bugs are transiently aposymbiotic (contain no symbionts), recombinant endosymbionts are able to effectively compete with other bacteria to establish

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press

infection when CRUZIGARD is ingested by early instar larvae (Durvasula *et al.*, 1999b). After homes are cleared of bugs with insecticides, treatment with CRUZIGARD is hoped to facilitate establishment of a disease-refractory population that could limit the need for repeated insecticide application (Beard *et al.*, 1998).

Of the possible drive mechanisms, only the use of CRUZIGARD has been seriously investigated experimentally in a transgenic insect intended for deployment in nature. Given the existence of multiple species with transgenic lines expressing GFP, empirical investigations of drive mechanisms can and should proceed rather than be postponed until transformed species are ready for deployment. The relative lack of attention focused on solving this problem threatens to offset the advances being made in the laboratory to develop transgenic insects suitable for release because the mechanism ultimately chosen for driving a gene through a population may determine in whole or in part the transformation techniques that are appropriate.

Potential risks associated with environmental release

Nearly all research on transgenic arthropods has focused on technical hurdles in the laboratory. Relatively little work has been done to determine how best to evaluate either intentional or accidental field releases, let alone actually to perform such evaluations. Uniform, international containment guidelines for research involving transgenic arthropods are lacking (Hoy *et al.*, 1997), and what guidelines do exist generally focus on preventing the spread of human pathogens (The American Committee of Medical Entomology of the American Society of Tropical Medicine and Hygiene, 2002; National Institutes of Health, 2002). Research laboratory containment procedures for transgenic arthropods that pose no human health risks would minimize but may not completely eliminate unintentional escapes. The extreme mobility, small size and large number of transgenic *Drosophila* that exist in laboratories around the world raise the question of whether accidental releases of this species have occurred. None have been reported in the literature, but scant attention has been given to what the impact may be, and their detection without actively monitoring for them would be extremely unlikely. However, even if some escapes have occurred, most transgenic *Drosophila* are less fit than their wild-type counterparts, often requiring special rearing conditions in the laboratory that would preclude their establishment in the field or successful competition with any wild conspecifics. Nevertheless, certain introduced traits such as insecticide resistance (Benedict *et al.*, 1994) do raise concerns over the possibility of unintentional introductions even for arthropods that pose no threat to public health (Hoy *et al.*, 1997). In addition, it may be time to consider whether it would be wise to follow this model for the increasing number of other insect genera that are being transformed worldwide in research laboratories.

The assumption that transgenic organisms have reduced fitness may not be valid for insects that are being engineered to replace natural populations. Existing risk assessment paradigms for genetically-modified organisms deal with the difficulties of containing them and define risk to be the probability that a transgene will escape into nature times the hazard that it would cause (Muir & Howard, 2002). Currently no established procedures exist for how a risk assessment for transgenic insects would be conducted when the goal is in fact to spread the transgene as widely as possible (Hoy, 1995, 2000). Whether the risks associated with transgenic insects are unique in kind or greater in degree than those associated with the release of insects that have been genetically altered by more conventional methods such as translocations or irradiation is questionable. The release of insects that have been genetically altered by conventional methods has occurred worldwide for many years (Braig & Yan, 2002). However, as with agricultural crops (National Research Council, 2002), genetic transformation of insects has raised the call for greater regulatory oversight.

A detailed list has been published of the issues that should be considered when evaluating the risks and probability of success for deployment of a population of genetically-modified insects into experimental field plots (Hoy, 2000). These issues include attributes of the unmodified organism (e.g. What is its potential for gene flow with other organisms? What role does it play in the ecosystem? What is its mobility, longevity, and reproductive potential?), attributes of the genetic alteration (e.g. What is its nature, function, and stability?), attributes of the accessible environment (e.g. Is the arthropod already established in the location? Are there alternative hosts or prey? Are agents of indirect dissemination present?), and a comparison of the modified and unmodified phenotype. These issues will be even more difficult to resolve for uncontained releases given the size of the reasonable dispersal area for any insect and the multitude of biological interactions that would thus occur. Their resolution will, however, be especially important for evaluating the most important potential risk issues that are discussed below.

Genetic exchange with natural, conspecific populations

Genetic variation in natural populations would usually be much greater than that in a strain of transgenic insects because these strains experience severe bottlenecks during production. Experiments do suggest that establishing highly outbred transgenic populations would have substantial fitness benefits for the introduced individuals, but considerably more work is involved (Catteruccia *et al.*, 2003). In the absence of such measures, mating between transgenics and wild conspecifics would expose the transgenes to novel genetic backgrounds that could allow for a broad range of non-additive effects. These gene interactions may increase vector competence, alter ecological requirements that could expand vector host range, or affect life-history characteristics that would indirectly increase vector competence by affecting survival or reproductive output (Gubler, 1993). Given insects' small size, high reproductive potential, and dispersal ability, even accidental releases may lead to gene flow with wild populations. Investigations of insecticide resistance in *Drosophila* have revealed that novel traits may spread over huge geographic distances in remarkably short periods of time (Daborn *et al.*, 2002). Releases may also be recurrent events that would provide a continual source of transgenes such that their persistence would be independent of their fitness effects. The risk associated with the (unintended) spread of a transgene through a natural population will depend on the trait(s) conferred by the gene of interest and the marker gene, as well as their expression patterns as

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press

modulated by the promoter. Anti-microbials are predominant among the traits currently being contemplated for insects, and these could have potentially dramatic impacts on recipient organisms, depending on the specificity of the anti-microbial activity.

Horizontal gene transfer

The potential of a transgene to move horizontally in addition to vertically should be considered when assessing the risks of any release of transgenic arthropods. Some of the clearest examples of horizontal gene transfer have occurred with transposable elements in insects, and some of these events have occurred so recently that the phenomenon appears to be of concern even over a relatively short rather than simply evolutionary timescale for closely-related organisms. Genetic and molecular evidence suggests that the hobo transposable element spread worldwide through natural populations of D. melanogaster in the last 50 years (Pascual & Periquet, 2003). Several complementary lines of evidence indicate that P elements have probably spread through D. melanogaster within the last 50 years as well (Kidwell, 1992). Individuals recently collected from wild populations around the world have all been found to have P elements, but most individuals from strains that had been maintained in laboratories for at least 20 years lacked them (Anxolabéhère et al., 1988). Sequence data also provides evidence for the recent invasion of P elements, as elements from distant geographical locations have extreme sequence similarity (Kidwell, 1992). In addition, laboratory experiments have demonstrated the ability of P elements to spread rapidly through populations in which they were previously absent (Kidwell et al., 1981; Good et al., 1989). Interspecific horizontal transfer among Drosophila is also well supported for P elements (Kiyasu & Kidwell, 1984; Stacey et al., 1986; Daniels et al., 1990; Haring et al., 2000; Silva & Kidwell, 2000). In addition, the transposable element mariner is believed to have spread by horizontal transfer between drosophilids in different genera (Maruyama & Hartl, 1991) as well as between insects of different orders (Robertson & Lampe, 1995).

The potential for horizontal gene transfer should be considered also when evaluating releases of transgenic gut microorganisms. The transfer of genes among bacteria, including those in different genera has been well documented (Davies, 1994), and sequencing of bacterial genomes continues to provide additional support for this phenomenon (Pennisi, 1998). An analysis of the entire genome sequence for *Escherichia coli* (Enterobacteriaceae) revealed that at least 17.6% of the genome had originated from horizontal transfer (Lawrence & Ochman, 1998). Little is known about how gut symbionts themselves are transferred horizontally, but it appears to be frequent for *Wolbachia* on both an evolutionary time scale (Heath *et al*., 1999) and over a short time frame in certain circumstances, e.g. among wasp larvae sharing a common food source (Huigens *et al*., 2000).

Surprisingly little is understood about the process and mechanism of gene transfer. It is not known, for example, how frequently horizontal transfer takes place by even a rough estimate (Kidwell, 1992), and little more is known about the mechanism(s) by which it occurs. Several possible avenues for horizontal gene transfer among insects have been proposed including bacterial symbionts and parasites (Houck *et al*., 1991). Evidence that baculoviruses are able to incorporate genes from their host genome (Fraser *et al*., 1995) has also raised the idea that they may be a route of transfer, but none of these possibilities has been convincingly demonstrated (Capy *et al*., 1994). Given this uncertainty, assessment of the potential harm that could result if horizontal gene transfer were to occur for a specific transgene would be particularly important. Risk assessment for release of transgenic insects will be complicated by the inability to judge the probability that horizontal gene transfer may occur unless the level of harm that might result can be determined to be acceptably low.

Instability of introduced genes

Transposable elements are found in most organisms and can account for a large portion of an organism's genome (Kidwell & Lisch, 1997). There is direct and indirect evidence that certain transposable elements may have the ability to crossmobilize, i.e. to enable mobility in related transposable elements. Crossmobilization could jeopardize the stability of any introduced gene in a host with a transposable element from the same family as that used in the vector (O'Brochta & Atkinson, 1996). *Hermes* and *hobo* are members of the same *hAT* family of transposable elements and have been shown to be capable of crossmobilization even though their transposase coding regions are only 55% identical at the amino acid level (Sundararajan *et al*., 1999). Stability of the inserted genes is necessary to ensure that a released population will in fact carry the desired trait. For applications of transgenic arthropods that would involve releases into nature of organisms that would be serious pests were it not for the presence of the transgenes, stability of the insert is necessary to ensure that the released population will pose no hazards to human health, agriculture, or the environment.

Potential instability in transformed populations is not limited to those that are modified by use of transposable elements. The experimental release of transgenic *M. occidentalis* (modified by illegitimate recombination) had the surprising result that after only three generations, very few individuals actually contained the transgene, in spite of its persistence in laboratory strains for over 150 generations. An additional experiment confirmed that the transgene was rapidly lost under field conditions (Hoy, 2000). Such results emphasize the importance of contained semi-field experiments over many generations to evaluate the stability of transgene inserts in any population that might be released in the wild. Although such experiments must necessarily be done with the actual population intended for release, experiments could be conducted now using marker genes to evaluate strategies that may minimize the potential for loss of transgene expression.

Introduction of pests to human-populated areas

The intentional release of transgenic insects may affect human populations in ways and to a degree not seen before with transgenic agricultural products. Insects intended for intentional release to reduce disease vector ability will need, at some stage, to undergo field trials in areas where humans are normally exposed to the pathogens transmitted by these vectors (Aultman *et al*., 2000). Given the high mobility and fecundity of insects, even a localized environmental release

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press

could potentially impact a large geographic region.

Even under the best-case scenario in which the introduced construct behaves exactly as anticipated and successfully sweeps through the local arthropod population, the release of human parasites would be associated with potential human health risks that should be carefully considered and addressed before deployment (Spielman *et al*., 2002). For example, *A. aegypti* is capable of transmitting viral pathogens including yellow fever, dengue and chikungunya as well as a number of filarial parasites (Adelman *et al*., 2002). It is adapted to living in close proximity to human populations and feeds almost entirely on human blood. Any introduced population of this species could thus potentially transmit several human diseases unless specifically engineered to be refractory to all of them. Not only would the transformed insects be introduced, perhaps repeatedly over a wide area, but the population might need to be actively supported so that it could successfully establish in the region (Spielman, 1994). The liability and risk assessment issues with such a scenario are extremely complicated.

In some areas, multiple malaria vectors occur sympatrically. The effects of a control programme that focused on only one species would have to take into consideration the effect this would have on the other vectors in the region. Knowledge of population dynamics and species interactions would be needed to verify that modification of one population would have the desired effect on overall disease transmission. Likewise, the diversity of malaria parasites must be considered to determine whether the engineered mosquitoes would be equally refractory to all potential parasites and to ensure that the genetic diversity in the parasite population would not allow immediate evolution of a strain resistant to the control measure. In practice, multiple parasite-inhibiting mechanisms may need to be incorporated into the mosquitoes to prevent such a scenario (Braig & Yan, 2002).

Conclusions

Arthropods present numerous challenges to agricultural production and to public health. Biotechnology holds great promise for producing tools that would enable scientists to address these issues in novel ways that may prove to be not only more effective but also safer to human and environmental health. These advantages of transformation technology may not, however, be taken for granted. Although remarkable progress has been made in arthropod genetic transformation in the laboratory, these advances have not been accompanied by many relevant experimental evaluations of techniques for driving a gene through a population or many comprehensive risk assessments of the potential effects of deploying transgenic arthropods in the field. When evaluating risks and benefits, a silkworm modified with a baculovirus to improve silk quality in contained production systems is expected to have little in common with a mosquito modified with a transposable element to express an anti-microbial for release into nature. Molecular biologists who are responsible for the recent scientific advances are unlikely also to become the experts in population genetics and ecology who are needed to address the legitimate issues confronting use of this technology. Greater collaboration among scientists across disciplines could facilitate evaluations of the most relevant issues remaining before deployment. In the case of arthropod transgenesis, the most difficult and sensitive issues are likely to present themselves early on in the process at a point at which little regulatory experience will exist to guide decision-making and policy development. Experience with agricultural biotechnology may serve as a warning as to what may occur if the public perceives science has proceeded more quickly than our ability to understand its ramifications or to sufficiently control its risks.

Acknowledgements

The author thanks P.W. Atkinson, Tom McClintock, Elizabeth Milewski, Guinnevere Roberts and two anonymous reviewers for helpful comments on the manuscript.

References

Adelman, Z.N., Jasinskiene, N. & James, A.A. (2002) Development and applications of transgenesis in the yellow fever mosquito, *Aedes aegypti*. *Molecular and Biochemical Parasitology* **121**, 1–10.

Afanasiev, B.N. & Carlson, J.C. (2000) Densovirinae as gene transfer vehicles. *Contributions to Microbiology* **4**, 33–58.

Afanasiev, B.N., Kozlov, Y.V., Carlson, J.O. & Beaty, B.J. (1994) Densovirus of *Aedes aegypti* as an expression vector in mosquito cells. *Experimental Parasitology* **79**, 322–339.

Afanasiev, B.N., Ward, T.W., Beaty, B.J. & Carlson, J.C. (1999) Transduction of *Aedes aegypti* mosquitoes with vectors derived from *Aedes* densovirus. *Virology* **257**, 62–72.

Aksoy, S. (2000) Tsetse – a haven for microorganisms. *Parasitology Today* **16**, 114–118.

Aksoy, S. (2003) Control of tsetse flies and trypanosomes using molecular genetics. *Veterinary Parasitology* **115**, 125–145.

Aksoy, S., Maudlin, I., Dale, C., Robinson, A.S. & O'Neill, S.L. (2001) Prospects for control of African trypanosomiasis by tsetse vector manipulation. *Trends in Parasitology* **17**, 29–35.

Allen, M.L., O'Brochta, D.A., Atkinson, P.W. & Levesque, C.S. (2001) Stable, germ-line transformation of *Culex quinquefasciatus* (Diptera: Culicidae). *Journal of Medical Entomology* **38**, 701–710.

Allen-Miura, T., Afanasiev, B.N., Olson, K.E., Beaty, B.J. & Carlson, J.C. (1999) Packaging of AeDNV-GFP transducing virus by expression of densovirus structural proteins from a sindbis virus expression system. *Virology* **257**, 54–61.

Anxolabéhère, D., Kidwell, M.G. & Periquet, G. (1988) Molecular characteristics of diverse populations are consistent with the hypothesis of a recent invasion of *Drosophila melanogaster* by mobile *P* elements. *Molecular Biology and Evolution* **5**, 252–269.

Atkinson, P.W. & James, A.A. (2002) Germline transformants spreading out to many insect species. *Advances in Genetics* **47**, 49–86.

Aultman, K.S., Walker, E.D., Gifford, F., Severson, D.W., Beard, C.B. & Scott, T.W. (2000) Managing risks of arthropod vector research. *Science* **288**, 2321–2322.

Barreau, C., Jousset, F.X. & Cornet, M. (1994) An efficient and easy method of infection of mosquito larvae from virus-contaminated cell cultures. *Journal of Virological Methods* **49**, 153–156.

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press

Beard, C.B., Mason, P.W., Aksoy, S., Tesh, R.B. & Richards, F.F. (1992) Transformation of an insect symbiont and expression of a foreign gene in the Chagas' disease vector *Rhodnius prolixus*. *American Journal of Tropical Medicine and Hygiene* **46**, 195–200.

Beard, C.B., Durvasula, R.V. & Richards, F.F. (1998) Bacterial symbiosis in arthropods and the control of disease transmission. *Emerging Infectious Diseases* **4**, 581–591.

Beaty, B.J. (2000) Genetic manipulation of vectors: a potential novel approach for control of vector-borne diseases. *Proceedings of the National Academy of Sciences, USA* **97**, 10295–10297.

Benedict, M.Q., Scott, M.J. & Cockburn, A.F. (1994) High-level expression of the bacterial *opd* gene in *Drosophila melanogaster*: improved inducible insecticide resistance. *Insect Molecular Biology* **3**, 247–252.

Benedict, M.Q., Salazar, C.E. & Collins, F.H. (1995) A new dominant selectable marker for genetic transformation; *Hsp70-opd*. *Insect Biochemistry and Molecular Biology* **25**, 1061–1065.

Berghammer, A.J., Klingler, M. & Wimmer, E.A. (1999) A universal marker for transgenic insects. *Nature* **402**, 370.

Blackman, R.K., Koehler, M.M., Grimaila, R. & Gelbar, W.M. (1989) Identification of a fully-functional *hobo* transposable element and its use for germ-line transformation of *Drosophila*. *EMBO Journal* **8**, 211–217.

Braig, H.R. & Yan, G. (2002) The spread of genetic constructs in natural insect populations. pp. 251–314 *in* Letourneau, D.K. & Burrows, B.E. (*Eds*) *Genetically engineered organisms: assessing environmental and human health effects*. Boca Raton, Florida, CRC Press.

Brookfield, J.F.Y. (1986) The population biology of transposable elements. *Philosophical Transactions of the Royal Society of London B* **312**, 217–226.

Capy, P., Anxolabéhère, D. & Langin, T. (1994) The strange phylogenies of transposable elements: are horizontal transfers the only explanation? *Trends in Genetics* **10**, 7–12.

Carareto, C.M., Kim, W., Wojciechowski, M.F., O'Grady, P., Prokchorova, A.V., Silva, J.C. & Kidwell, M.G. (1997) Testing transposable elements as genetic drive mechanisms using *Drosophila* P element constructs as a model system. *Genetica* **101**, 13–33.

Catteruccia, F., Nolan, T., Loukeris, T.G., Blass, C., Savakis, C., Kafatos, F.C. & Crisanti, A. (2000) Stable germline transformation of the malaria mosquito *Anopheles stephensi*. *Nature* **405**, 959–962.

Catteruccia, F., Godfray, H.C.J. & Crisanti, A. (2003) Impact of genetic manipulation on the fitness of *Anopheles stephensi* mosquitoes. *Science* **299**, 1225–1227.

Cheng, Q. & Aksoy, S. (1999) Tissue tropism, transmission and expression of foreign genes *in vivo* in midgut symbionts of tsetse flies. *Insect Molecular Biology* **8**, 125–132.

Coates, C.J., Turney, C.L., Frommer, M., O'Brochta, D.A. & Atkinson, P.W. (1997) Interplasmid transposition of the *mariner* transposable element in non-drosophilid insects. *Molecular and General Genetics* **253**, 728–733.

Coates, C.J., Jasinskiene, N., Miyashiro, L. & James, A.A. (1998) *Mariner* transposition and transformation of the yellow fever mosquito, *Aedes aegypti*. *Proceedings of the National Academy of Sciences, USA* **95**, 3748–3751.

Coates, C.J., Jasinskiene, N., Pott, G.B. & James, A.A. (1999) Promoter-directed expression of recombinant fire-fly luciferase in the salivary glands of *Hermes*-transformed *Aedes aegypti*. *Gene* **226**, 317–325.

Coates, C.J., Jasinskiene, N., Morgan, D., Tosi, L.R.O., Beverley, S.M. & James, A.A. (2000) Purified *mariner* (*Mos1*) transposase catalyzes the integration of marked elements into the germ-line of the yellow fever mosquito, *Aedes aegypti*. *Insect Biochemistry and Molecular Biology* **30**, 1003–1008.

Collins, F.H. & Paskewitz, S.M. (1995) Malaria: current and future prospects for control. *Annual Review of Entomology* **40**, 195–219.

Cornel, A.J., Benedict, M.Q., Rafferty, C.S., Howells, A.J. & Collins, F.H. (1997) Transient expression of the *Drosophila melanogaster cinnabar* gene rescues eye colour in the *white eye* (*WE*) strain of *Aedes aegypti*. *Insect Biochemistry and Molecular Biology* **27**, 993–997.

Crampton, J.M., Stowell, S.L., Karras, M. & Sinden, R.E. (1999) Model systems to evaluate the use of transgenic haematophagous insects to deliver protective vaccines. *Parassitologia* **41**, 473–477.

Curtis, C.F. (1968) Possible use of translocations to fix desirable genes in insect pest populations. *Nature* **218**, 368–369.

Curtis, C.F. (1994) The case for malaria control by genetic manipulation of its vectors. *Parasitology Today* **10**, 371–374.

Daborn, P.J., Yen, J.L., Bogwitz, M.R., Le Goff, G., Feil, E., Jeffers, S., Tijet, N., Perry, T., Heckel, D., Batterham, P., Feyereisen, R., Wilson, T.G. & ffrench-Constant, R.H. (2002) A single P450 allele associated with insecticide resistance in *Drosophila*. *Science* **297**, 2253–2256.

Dale, C. & Welburn, S.C. (2001) The endosymbionts of tsetse flies: manipulating host-parasite interactions. *International Journal for Parasitology* **31**, 628–631.

Daniels, S.B., Clark, S.H., Kidwell, M.G. & Chovnick, A. (1987) Genetic transformation of *Drosophila melanogaster* with an autonomous *P* element: phenotypic and molecular analyses of long-established transformed lines. *Genetics* **115**, 711–723.

Daniels, S.B., Peterson, K.R., Strausbaugh, L.D., Kidwell, M.G. & Chovnick, A. (1990) Evidence for horizontal transmission of the *P* transposable element between *Drosophila* species. *Genetics* **124**, 339–355.

Davies, J. (1994) Inactivation of antibiotics and the dissemination of resistance genes. *Science* **264**, 375–382.

de Lara Capurro, M., Coleman, J., Beerntsen, B.T., Myles, K.M., Olson, K.E., Rocha, E., Krettli, A.U. & James, A.A. (2000) Virus-expressed, recombinant single-chain antibody blocks sporozoite infection of salivary glands in *Plasmodium gallinaceum*-infected *Aedes aegypti*. *American Journal of Tropical Medicine and Hygiene* **62**, 427–433.

Durvasula, R.V., Gumbs, A., Panackal, A., Kruglov, O., Aksoy, S., Merrifield, R.B., Richards, F.F. & Beard, C.B. (1997) Prevention of insect-borne disease: an approach using transgenic symbiotic bacteria. *Proceedings of the National Academy of Sciences, USA* **94**, 3274–3278.

Durvasula, R.V., Gumbs, A., Panackal, A., Kruglov, O., Taneja, J., Kang, A.S., Cordon-Rosales, C., Richards, F.F., Whitham, R.G. & Beard, C.B. (1999a) Expression of a functional antibody fragment in the gut of *Rhodnius prolixus* via transgenic bacterial symbiont *Rhodococcus rhodnii*. *Medical and Veterinary Entomology* **13**, 115–119.

Durvasula, R.V., Kroger, A., Goodwin, M., Panackal, A., Kruglov, O., Taneja, J., Gumbs, A., Richards, F.F., Beard, C.B. & Cordon-Rosales, C. (1999b) Strategy for introduction of foreign genes into field populations of Chagas disease vectors. *Annals of the Entomological Society of America* **92**, 937–943.

Enserink, M. (2000) Building a disease-fighting mosquito. *Science* **290**, 440–441.

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press

Finnegan, D.J. (1989) Eukaryotic transposable elements and genome evolution. *Trends in Genetics* **5**, 103–107.

Franco, M., Rogers, M.E., Shimizu, C., Shike, H., Vogt, R.G. & Burns, J.C. (1998) Infection of lepidoptera with a pseudotyped retroviral vector. *Insect Biochemistry and Molecular Biology* **28**, 819–825.

Fraser, M.J., Cary, L., Boonvisudhi, K. & Wang, H.G. (1995) Assay for movement of lepidopteran transposon IFP2 in insect cells using a baculovirus genome as a target DNA. *Virology* **211**, 397–407.

Galindo, M.I., Ladevèze, V., Lemeunier, F., Kalmes, R., Periquet, G. & Pascual, L. (1995) Spread of the autonomous transposable element *hobo* in the genome of *Drosophila melanogaster*. *Molecular Biology and Evolution* **12**, 723–734.

Gardner, M.J., Hall, N., Fung, E., White, O., Berriman, M., Hyman, R.W., Carlton, J.M., Pain, A., Nelson, K.E., Bowman, S., Paulsen, I.T., James, K., Eisen, J.A., Rutherford, K., Salzberg, S.L., Craig, A., Kyes, S., Chan, M.S., Nene, V., Shallom, S.J., Suh, B., Peterson, J., Angiuoli, S., Pertea, M., Allen, J., Selengut, J., Haft, D., Mather, M.W., Vaidya, A.B., Martin, D.M., Fairlamb, A.H., Fraunholz, M.J., Roos, D.S., Ralph, S.A., McFadden, G.I., Cummings, L.M., Subramanian, G.M., Mungall, C., Venter, J.C., Carucci, D.J., Hoffman, S.L., Newbold, C., Davis, R.W., Fraser, C.M. & Barrell, B. (2002) Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* **419**, 498–511.

Giraud, C., Devauchelle, G. & Bergoin, M. (1992) The densovirus of *Junonia coenia* (Jc DNV) as an insect cell expression vector. *Virology* **186**, 207–218.

Good, A.G., Meister, G.A., Borck, H.W., Grigliatti, T.A. & Hickey, D.A. (1989) Rapid spread of transposable *P* elements in experimental populations of *Drosophila melanogaster*. *Genetics* **122**, 387–396.

Gorman, M.J., Severson, D.W., Cornel, A.J., Collins, F.H. & Paskewitz, S.M. (1997) Mapping a quantitative trait locus involved in melanotic encapsulation of foreign bodies in the malaria vector, *Anopheles gambiae*. *Genetics* **146**, 965–971.

Grossman, G.L., Rafferty, C.S., Clayton, J.R., Stevens, T.K., Mukabayire, O. & Benedict, M.Q. (2001) Germline transformation of the malaria vector, *Anopheles gambiae*, with the *piggyBac* transposable element. *Insect Molecular Biology* **10**, 597–604.

Gubler, D.J. (1993) Release of exotic genomes. *Journal of the American Mosquito Control Association* **9**, 104.

Hahn, C.S., Yahn, Y.S., Braciale, T.J. & Rice, C.M. (1992) Infectious Sindbis virus transient expression vectors for studying antigen processing and presentation. *Proceedings of the National Academy of Sciences, USA* **89**, 2679–2683.

Handler, A.M. & Harrell, R.A.I. (2001) Transformation of the Caribbean fruit fly, *Anastrepha suspensa*, with a *piggyBac* vector marked with polyubiquitin-regulated GFP. *Insect Biochemistry and Molecular Biology* **31**, 199–205.

Handler, A.M. & McCombs, S.D. (2000) The *piggyBac* transposon mediates germ-line transformation in the Oriental fruit fly and closely related elements in its genome. *Insect Molecular Biology* **9**, 605–612.

Handler, A.M., McCombs, S.D., Fraser, M.J. & Saul, S.H. (1998) The lepidopteran transposon vector, *piggyBac*, mediates germ-line transformation in the Mediterranean fruit fly. *Proceedings of the National Academy of Sciences, USA* **95**, 7520–7525.

Hao, Z., Kasumba, I., Lehane, M.J., Gibson, W.C., Kwon, J. & Aksoy, S. (2001) Tsetse immune responses and trypanosome transmission: implications for the development of tsetse-based strategies to reduce trypanosomiasis. *Proceedings of the National Academy of Sciences, USA* **98**, 12648–12653.

Haring, E., Hagemann, S. & Pinsker, W. (2000) Ancient and recent horizontal invasions of drosophilids by *P* elements. *Journal of Molecular Evolution* **51**, 577–586.

Heath, B.D., Butcher, R.D., Whitfield, W.G. & Hubbard, S.F. (1999) Horizontal transfer of *Wolbachia* between phylogenetically distant insect species by a naturally occurring mechanism. *Current Biology* **9**, 313–316.

Hediger, M., Niessen, M., Wimmer, E.A., Dübendorfer, A. & Bopp, D. (2001) Genetic transformation of the housefly *Musca domestica* with the lepidopteran derived transposon *piggyBac*. *Insect Molecular Biology* **10**, 113–119.

Heinrich, J.C., Li, X., Henry, R.A., Haack, N., Stringfellow, L., Heath, A.C.G. & Scott, M.J. (2002) Germ-line transformation of the Australian sheep blowfly *Lucilia cuprina*. *Insect Molecular Biology* **11**, 1–10.

Higgs, S., Rayner, J.O., Olson, K.E., Davis, B.S., Beaty, B.J. & Blair, C.D. (1998) Engineered resistance in *Aedes aegypti* to a West African and a South American strain of yellow fever virus. *American Journal of Tropical Medicine and Hygiene* **58**, 663–670.

Ho, W.K., Meng, Z.Q., Lin, H.R., Poon, C.T., Leung, Y.K., Yan, K.T., Dias, N., Che, A.P., Liu, J., Zheng, W.M., Sun, Y. & Wong, A.O. (1998) Expression of grass carp growth hormone by baculovirus in silkworm larvae. *Biochimica et Biophysica Acta* **1381**, 331–339.

Holt, R.A., Subramanian, G.M., Halpern, A., Sutton, G.G., Charlab, R., Nusskern, D.R., Wincker, P., Clark, A.G., Ribeiro, J.M., Wides, R., Salzberg, S.L., Loftus, B., Yandell, M., Majoros, W.H., Rusch, D.B., Lai, Z., Kraft, C.L., Abril, J.F., Anthouard, V., Arensburger, P., Atkinson, P.W., Baden, H., de Berardinis, V., Baldwin, D., Benes, V., Biedler, J., Blass, C., Bolanos, R., Boscus, D., Barnstead, M., Cai, S., Center, A., Chatuverdi, K., Christophides, G.K., Chrystal, M.A., Clamp, M., Cravchik, A., Curwen, V., Dana, A., Delcher, A., Dew, I., Evans, C.A., Flanigan, M., Grundschober-Freimoser, A., Friedli, L., Gu, Z., Guan, P., Guigo, R., Hillenmeyer, M.E., Hladun, S.L., Hogan, J.R., Hong, Y.S., Hoover, J., Jaillon, O., Ke, Z., Kodira, C., Kokoza, E., Koutsos, A., Letunic, I., Levitsky, A., Liang, Y., Lin, J.J., Lobo, N.F., Lopez, J.R., Malek, J.A., McIntosh, T.C., Meister, S., Miller, J., Mobarry, C., Mongin, E., Murphy, S.D., O'Brochta, D.A., Pfannkoch, C., Qi, R., Regier, M.A., Remington, K., Shao, H., Sharakhova, M.V., Sitter, C.D., Shetty, J., Smith, T.J., Strong, R., Sun, J., Thomasova, D., Ton, L.Q., Topalis, P., Tu, Z., Unger, M.F., Walenz, B., Wang, A., Wang, J., Wang, X., Woodford, K.J., Wortman, J.R., Wu, M., Yao, A., Zdobnov, E.M., Zhang, H., Zhao, S., Zhu, S.C., Zhimulev, I., Coluzzi, M., delle Torre, A., Roth, C.W., Louis, C., Kalush, F., Mural, R.J., Myers, E.W., Adams, M.D., Smith, H.O., Broder, S., Gardner, M.J., Fraser, C.M., Birney, E., Bork, P., Brey, P.T., Venter, J.C., Weissenbach, J., Kafatos, F.C., Collins, F.H. & Hoffman, S.L. (2002) The genome sequence of the malaria mosquito *Anopheles gambiae*. *Science* **298**, 129–149.

Houck, M.A., Clark, J.B., Peterson, K.R. & Kidwell, M.G. (1991) Possible horizontal transfer of *Drosophila* genes by the mite *Proctolaelaps regalis*. *Science* **253**, 1125–1128.

Hoy, M.A. (1995) Impact of risk analysis on pest-management programs employing transgenic arthropods. *Parasitology Today* **11**, 229–232.

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press

Hoy, M.A. (2000) Transgenic arthropods for pest management programs: risks and realities. *Experimental and Applied Acarology* **24**, 463–495.

Hoy, M.A., Gaskalla, R.D., Capinera, J.L. & Keierleber, C.N. (1997) Laboratory containment of transgenic arthropods. *American Entomologist* **43**, 206–256.

Huigens, M.E., Luck, R.F., Klaassen, R.H.G., Maas, M.F.P.M., Timmermans, M.J.T.N. & Stouthamer, R. (2000) Infectious parthenogenesis. *Nature* **405**, 178–179.

Ito, J., Ghosh, A.K., Moreira, L.A., Wimmer, E.A. & Jacobs-Lorena, M. (2002) Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. *Nature* **417**, 452–455.

Jacobson, J.W., Medhora, M.M. & Hartl, D.L. (1986) Molecular structure of a somatically unstable transposable element in *Drosophila*. *Proceedings of the National Academy of Sciences, USA* **83**, 8684–8688.

Jasinskiene, N., Coates, C.J., Benedict, M.Q., Cornel, A.J., Salazar Rafferty, C., James, A.A. & Collins, F.H. (1998) Stable transformation of the yellow fever mosquito, *Aedes aegypti*, with the *Hermes* element from the housefly. *Proceedings of the National Academy of Sciences, USA* **95**, 3743–3747.

Johanowicz, D.L. & Hoy, M.A. (1999) *Wolbachia* infection dynamics in experimental laboratory populations of *Metaseiulus occidentalis*. *Entomologia Experimentalis et Applicata* **93**, 259–268.

Johnson, B.W., Olson, K.E., Allen-Miura, T., Rayms-Keller, A., Carlson, J.O., Coates, C.J., Jasinskiene, N., James, A.A., Beaty, B.J. & Higgs, S. (1999) Inhibition of luciferase expression in transgenic *Aedes aegypti* mosquitoes by Sindbis virus expression of antisense luciferase RNA. *Proceedings of the National Academy of Sciences, USA* **96**, 13399–13403.

Jordan, T.V., Shike, H., Boulo, V., Cedeno, V., Fang, Q., Davis, B.S., Jacobs-Lorena, M., Higgs, S., Fryxell, K.J. & Burns, J.C. (1998) Pantropic retroviral vectors mediate somatic cell transformation and expression of foreign genes in dipteran insects. *Insect Molecular Biology* **7**, 215–222.

Kadonookuda, K., Yamamoto, M., Higashino, Y., Taniai, K., Kato, Y., Chowdhury, S., Xu, J.H., Choi, S., Sugiyama, M., Nakashima, K., Maeda, S. & Yamakawa, M. (1995) Baculovirus-mediated production of the human growth hormone in larvae of the silkworm, *Bombyx mori*. *Biochemical and Biophysical Research Communications* **213**, 389–396.

Kaminker, J.S., Bergman, C.M., Kronmiller, B., Carlson, J., Svirskas, R., Patel, S., Frise, E., Wheeler, D.A., Lewis, S.E., Rubin, G.M., Ashburner, M. & Celniker, S.E. (2002) The transposable elements of the *Drosophila melanogaster* euchromatin: a genomics perspective. *Genome Biology* **3**, 0084.1–0084.20.

Karpf, A.R., Lenches, E., Strauss, E.G., Strauss, J.H. & Brown, D.T. (1997) Superinfection exclusion of alphaviruses in three mosquito cell lines persistently infected with Sindbis virus. *Journal of Virology* **71**, 7119–7123.

Kidwell, M.G. (1992) Horizontal transfer of P elements and other short inverted repeat transposons. *Genetica* **86**, 275–286.

Kidwell, M.G. & Lisch, D. (1997) Transposable elements as sources of variation in animals and plants. *Proceedings of the National Academy of Sciences, USA* **94**, 7704–7711.

Kidwell, M.G. & Ribeiro, J.M. (1992) Can transposable elements be used to drive disease refractoriness genes into vector populations? *Parasitology Today* **8**, 325–329.

Kidwell, M.G. & Wattam, A.R. (1998) An important step forward in the genetic manipulation of mosquito vectors of human disease. *Proceedings of the National Academy of Sciences, USA* **95**, 3349–3350.

Kidwell, M.G., Novy, J.B. & Feeley, S.M. (1981) Rapid unidirectional change of hybrid dysgenesis potential in *Drosophila*. *Journal of Heredity* **72**, 32–38.

Kimura, K. (2001) Transposable element-mediated transgenesis in insects beyond *Drosophila*. *Journal of Neurogenetics* **15**, 179–192.

Kiyasu, P.K. & Kidwell, M.G. (1984) Hybrid dysgenesis in *Drosophila melanogaster*: the evolution of mixed *P* and *M* populations maintained at high temperature. *Genetical Research* **44**, 251–259.

Kokoza, V., Ahmed, A., Cho, W., Jasinskiene, N., James, A.A. & Raikhel, A. (2000) Engineering blood meal-activated systemic immunity in the yellow fever mosquito, *Aedes aegypti*. *Proceedings of the National Academy of Sciences, USA* **97**, 9144–9149.

Ladevèze, V., Galindo, I., Chaminade, N., Pascual, L., Periquet, G. & Lemeunier, F. (1998) Transmission pattern of *hobo* transposable element in transgenic lines of *Drosophila melanogaster*. *Genetical Research* **71**, 97–107.

Ladevèze, V., Aulard, S., Chaminade, N., Biemont, C., Periquet, G. & Lemeunier, F. (2001) Dynamics of the *hobo* transposable element in transgenic lines of *Drosophila melanogaster*. *Genetical Research* **77**, 135–142.

Lawrence, J.G. & Ochman, H. (1998) Molecular archaeology of the *Escherichia coli* genome. *Proceedings of the National Academy of Sciences, USA* **95**, 9413–9417.

Liao, G.C., Rehm, E.J. & Rubin, G.M. (2000) Insertion site preferences of the *P* transposable element in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences, USA* **97**, 3347–3351.

Lidholm, D., Lohe, A.R. & Hartl, D.L. (1993) The transposable element *mariner* mediates germline transformation in *Drosophila melanogaster*. *Genetics* **134**, 859–868.

Lindquist, A.W. (1955) The use of gamma radiation for control or eradication of the screw-worm. *Journal of Economic Entomology* **48**, 467–469.

Lohe, A.R., Moriyama, E.N., Lidholm, D.A. & Hartl, D.L. (1995) Horizontal transmission, vertical inactivation, and stochastic loss of *mariner*-like transposable elements. *Molecular Biology and Evolution* **12**, 62–72.

Loukeris, T.G., Livadaras, I., Arcà, B., Zabalou, S. & Savakis, C. (1995) Gene transfer into the Medfly, *Ceratitis capitata*, with a *Drosophila hydei* transposable element. *Science* **270**, 2002–2005.

Lozovskaya, E.R., Nurminsky, D.I., Hartl, D.L. & Sullivan, D.T. (1996) Germline transformation of *Drosophila virilis* mediated by the transposable element *hobo*. *Genetics* **142**, 173–177.

Maruyama, K. & Hartl, D.L. (1991) Evidence for interspecific transfer of the transposable element *mariner* between *Drosophila* and *Zaprionus*. *Journal of Molecular Evolution* **33**, 514–524.

Meister, G.A. & Grigliatti, T.A. (1993) Rapid spread of a *P* element/*Adh* gene construct through experimental populations of *Drosophila melanogaster*. *Genome* **36**, 1169–1175.

Michel, K., Stamenova, A., Pinkerton, A.C., Franz, G., Robinson, A.S., Gariou-Papalexiou, A., Zacharopoulou, A., O'Brochta, D.A. & Atkinson, P.W. (2001) *Hermes*-mediated germ-line transformation of the Mediterranean

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press

fruit fly *Ceratitis capitata*. *Insect Molecular Biology* **10**, 155–162.

Moreira, L.A., Edwards, M.J., Adhami, F., Jasinskiene, N., James, A.A. & Jacobs-Lorena, M. (2000) Robust gut-specific gene expression in transgenic *Aedes aegypti* mosquitoes. *Proceedings of the National Academy of Sciences, USA* **97**, 10895–10898.

Mori, H. & Tsukada, M. (2000) New silk protein: modification of silk protein by gene engineering for production of biomaterials. *Reviews in Molecular Biotechnology* **74**, 95–103.

Mori, H., Yamao, M., Nakazawa, H., Sugahara, H., Shirai, N., Matsubara, F., Sumida, M. & Imamura, T. (1995) Transovarian transmission of a foreign gene in the silkworm, *Bombyx mori*, by *Autographa californica* nuclear polyhedrosis virus. *Bio/Technology* **13**, 1005–1007.

Muir, W.M. & Howard, R.D. (2002) Assessment of possible ecological risks and hazards of transgenic fish with implications for other sexually reproducing organisms. *Transgenic Research* **11**, 101–114.

National Institutes of Health (2002) Guidelines for research involving recombinant DNA molecules. *http://www4.od.nih.gov/oba/rac/guidelines_02/NIH_Guidelines_Apr_02.htm#_Toc7261556*

National Research Council (2002) *Environmental effects of transgenic plants: the scope and adequacy of regulation*. 320 pp. Washington, DC, National Academy Press.

Nolan, T., Bower, T.M., Brown, A.E., Crisanti, A. & Catteruccia, F. (2002) *piggyBac*-mediated germline transformation of the malaria mosquito *Anopheles stephensi* using the red fluorescent protein dsRED as a selectable marker. *Journal of Biological Chemistry* **277**, 8759–8762.

O'Brochta, D.A. & Atkinson, P.W. (1996) Transposable elements and gene transformation in non-drosophilid insects. *Insect Biochemistry and Molecular Biology* **26**, 739–753.

O'Brochta, D.A., Warren, W.D., Saville, K.J. & Atkinson, P.W. (1994) Interplasmid transposition of *Drosophila hobo* elements in non-drosophilid insects. *Molecular and General Genetics* **244**, 9–14.

O'Brochta, D.A., Atkinson, P.W. & Lehane, M.J. (2000) Transformation of *Stomoxys calcitrans* with a *Hermes* gene vector. *Insect Molecular Biology* **9**, 531–538.

O'Brochta, D.A., Warren, W.D., Saville, K.J. & Atkinson, P.W. (1996) *Hermes*, a function non-drosophilid insect gene vector from *Musca domestica*. *Genetics* **142**, 907–914.

O'Hare, K. & Rubin, G.M. (1983) Structures of *P* transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. *Cell* **34**, 25–35.

Olson, K.E., Higgs, S., Gaines, P.J., Powers, A.M., Davis, B.S., Kamrud, K.I., Carlson, J.O., Blair, C.D. & Beaty, B.J. (1996) Genetically engineered resistance to Dengue virus transmission in mosquitoes. *Science* **272**, 884–886.

Olson, K.E., Myles, K.M., Seabaugh, R.C., Higgs, S., Carlson, J.O. & Beaty, B.J. (2000) Development of a Sindbis virus expression system that efficiently expresses green fluorescent protein in midguts of *Aedes aegypti* following *per os* infection. *Insect Molecular Biology* **9**, 57–65.

Pascual, L. & Periquet, G. (2003) Distribution of *hobo* transposable elements in natural populations of *Drosophila melanogaster*. *Molecular Biology and Evolution* **8**, 282–296.

Peloquin, J.J., Thibault, S.T., Staten, R. & Miller, T.A. (2000) Germ-line transformation of pink bollworm (Lepidoptera: Gelechiidae) mediated by the *piggyBac* transposable element. *Insect Molecular Biology* **9**, 323–333.

Pennisi, E. (1998) Versatile gene uptake system found in cholera bacterium. *Science* **280**, 521–522.

Perera, O.P., Harrell, R.A.I. & Handler, A.M. (2002) Germ-line transformation of the South American malaria vector, *Anopheles albimanus*, with a *piggyBac/EGFP* transposon vector is routine and highly efficient. *Insect Molecular Biology* **11**, 291–297.

Pierro, D.J., Myles, K.M., Foy, B.D., Beaty, B.J. & Olson, K.E. (2003) Development of an orally infectious Sindbis virus transducing system that efficiently disseminates and expresses green fluorescent protein in *Aedes aegypti*. *Insect Molecular Biology* **12**, 107–116.

Pinkerton, A.C., O'Brochta, D.A. & Atkinson, P.W. (1996) Mobility of *hAT* transposable elements in the Old World bollworm, *Helicoverpa armigera*. *Insect Molecular Biology* **5**, 223–227.

Pinkerton, A.C., Michel, K., O'Brochta, D.A. & Atkinson, P.W. (2000) Green fluorescent protein as a genetic marker in transgenic *Aedes aegypti*. *Insect Molecular Biology* **9**, 1–10.

Powers, A.M., Kamrud, K.I., Olson, K.E., Higgs, S., Carlson, J.O. & Beaty, B.J. (1996) Molecularly engineered resistance to California serogroup virus replication in mosquito cells and mosquitoes. *Proceedings of the National Academy of Sciences, USA* **93**, 4187–4191.

Presnail, J.K. & Hoy, M.A. (1996) Maternal microinjection of the endoparasitoid *Cardiochiles diaphaniae* (Hymenoptera: Braconidae). *Annals of the Entomological Society of America* **89**, 576–580.

Presnail, J.K., Jeyaprakash, A., Li, J. & Hoy, M.A. (1997) Genetic analysis of four lines of *Metaseiulus occidentalis* (Acari: Phytoseiidae) transformed by maternal microinjection. *Annals of the Entomological Society of America* **90**, 237–245.

Preston, C.R. & Engels, W.R. (1989) Spread of P transposable elements in inbred lines of *Drosophila melanogaster*. *Progress in Nucleic Acid Research and Molecular Biology* **36**, 71–85.

Ribeiro, J.M. & Kidwell, M.G. (1994) Transposable elements as population drive mechanisms: specification of critical parameter values. *Journal of Medical Entomology* **31**, 10–16.

Robertson, H.M. (1993) The *mariner* transposable element is widespread in insects. *Nature* **362**, 241–245.

Robertson, H.M. & Lampe, D.J. (1995) Recent horizontal transfer of a *mariner* transposable element among and between Diptera and Neuroptera. *Molecular Biology and Evolution* **12**, 850–862.

Robinson, K.O., Ferguson, H.J., Cobey, S., Vaessin, H. & Smith, B.H. (2000) Sperm-mediated transformation of the honey bee, *Apis mellifera*. *Insect Molecular Biology* **9**, 625–634.

Rubin, G.M. & Spradling, A.C. (1982) Genetic transformation of *Drosophila* with transposable element vectors. *Science* **218**, 348–353.

Rubin, E., Lithwick, G. & Levy, A.A. (2001) Structure and evolution of the hAT transposon superfamily. *Genetics* **158**, 949–957.

Severson, D.W., Thathy, V., Mori, A., Zhang, Y. & Christensen, B.M. (1995) Restriction fragment length polymorphism mapping of quantitative trait loci for malaria parasite susceptibility in the mosquito *Aedes aegypti* . *Genetics* **139**, 1711–1717.

Silva, J.C. & Kidwell, M.G. (2000) Horizontal transfer and selection in the evolution of *P* elements. *Molecular Biology and Evolution* **17**, 1542–1557.

Spielman, A. (1994) Why entomological antimalaria research should not focus on transgenic mosquitoes. *Parasitology Today* **10**, 374–376.

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press

Spielman, A., Beier, J.C. & Kiszewski, A.E. (2002) Ecological and community considerations in engineering arthropods to suppress vector-borne disease. pp. 315–329 *in* Letourneau, D.K. & Burrows, B.E. (*Eds*) *Genetically engineered organisms: assessing environmental and human health effects*. Boca Raton, Florida, CRC Press.

Spradling, A.C. & Rubin, E. (1982) Transposition of cloned *P* elements into *Drosophila* germ line chromosomes. *Science* **218**, 341–347.

Stacey, S.N., Lansman, R.A., Brock, H.W. & Grigliatti, T.A. (1986) Distribution and conservation of mobile elements in the genus *Drosophila*. *Molecular Biology and Evolution* **3**, 522–534.

Strauss, E.G., Rice, C.M. & Strauss, J.H. (1984) Complete nucleotide sequence of the genomic RNA of Sindbis virus. *Virology* **133**, 92–110.

Sumitani, M., Yamamoto, D.S., Oishi, K., Lee, J.M. & Hatakeyama, M. (2003) Germline transformation of the sawfly, *Athalia rosae* (Hymenoptera: Symphyta), mediated by a *piggyBac*-derived vector. *Insect Biochemistry and Molecular Biology* **33**, 449–458.

Sundararajan, P., Atkinson, P.W. & O'Brochta, D.A. (1999) Transposable element interactions in insects: crossmobilization of *hobo* and *Hermes*. *Insect Molecular Biology* **8**, 359–368.

The American Committee of Medical Entomology of the American Society of Tropical Medicine and Hygiene (2002) Arthropod containment guidelines (version 3.1).

Toshiki, T., Chantal, T., Corinne, R., Toshio, K., Eappen, A., Mari, K., Natuo, K., Jean-Luc, T., Bernard, M., Gérard, C., Paul, S., Malcolm, F., Jean-Claude, P. & Pierre, C. (2000) Germline transformation of the silkworm *Bombyx mori* L. using a *piggyBac* transposon-derived vector. *Nature Biotechnology* **18**, 81–84.

Tsien, R.Y. (1998) The green fluorescent protein. *Annual Review of Biochemistry* **67**, 509–544.

Turelli, M. & Hoffmann, A.A. (1999) Microbe-induced cytoplasmic incompatibility as a mechanism for introducing transgenes into arthropod populations. *Insect Molecular Biology* **8**, 243–255.

Ward, T.W., Jenkins, M.S., Afanasiev, B.N., Edwards, M.J., Duda, B.A., Suchman, E., Jacobs-Lorena, M., Beaty, B.J. & Carlson, J.O. (2001) *Aedes aegypti* transducing densovirus pathogenesis and expression in *Aedes aegypti* and *Anopheles gambiae* larvae. *Insect Molecular Biology* **10**, 397–405.

Yamao, M., Katayama, N., Nakazawa, H., Yamakawa, M., Hayashi, Y., Hara, S., Kamei, K. & Mori, H. (2002) Gene targeting in the silkworm by use of a baculovirus. *Genes and Development* **13**, 511–516.

Yang, G., Chen, Z., Cui, D., Li, B. & Wu, X. (2002) Production of recombinant human calcitonin from silkworm (*B. mori*) larvae infected by baculovirus. *Current Pharmaceutical Design* **9**, 323–329.

Yoshiyama, M., Honda, H. & Kimura, K. (2000) Successful transformation of the housefly, *Musca domestica* (Diptera: Muscidae) with the transposable element, *mariner*. *Applied Entomology and Zoology* **35**, 321–325.

Zheng, L., Cornel, A.J., Wang, R., Erfle, H., Voss, H., Ansorge, W., Kafatos, F.C. & Collins, F.H. (1997) Quantitative trait loci for refractoriness of *Anopheles gambiae* to *Plasmodium cynomolgi* B. *Science* **276**, 425–428.

(Accepted 16 December 2003)
© US Government

https://doi.org/10.1079/BER2003290 Published online by Cambridge University Press