

ORIGINAL PAPER

C. J. Coates · C. L. Turney · M. Frommer
D. A. O'Brochta · P. W. Atkinson

Interplasmid transposition of the *mariner* transposable element in non-drosophilid insects

Received: 2 May 1996 / Accepted: 24 September 1996

Abstract Plasmid-based transposition assays were performed in developing embryos of the Australian sheep blowfly *Lucilia cuprina* and the Queensland fruit fly *Bactrocera tryoni*, using the *mariner* transposable element from *Drosophila mauritiana*. Transposition products were recovered that were identical in structure to those recovered from *D. melanogaster*. Only sequences delimited by the *mariner* terminal repeats were transposed and all insertions occurred at TA residues, and duplicated these. These are the hallmarks of *mariner* transpositions observed in the chromosomes of *D. melanogaster* and *D. mauritiana*, indicating that the plasmid-based assays are accurate indicators of *mariner* transposition activity. The recovery of precise transposition products from *L. cuprina* and *B. tryoni* demonstrates that *mariner* should be capable of producing germline transformants in these species. The results obtained from these assays suggests that they will be extremely useful in determining if *mariner* can transpose in other non-drosophilid insects and for investigating factors that might affect *mariner* transposition frequency.

Key words *Bactrocera tryoni* · *Drosophila melanogaster* · *Lucilia cuprina* · *Mariner* · Transposition assay

Communicated by D. J. Finnegan

C. J. Coates¹ · P. W. Atkinson² (✉)
Commonwealth Scientific and Industrial Research Organisation,
Division of Entomology, GPO Box 1700, Canberra ACT 2601,
Australia

C. L. Turney · M. Frommer
Fruit Fly Research Centre, School of Biological Sciences A12,
University of Sydney, NSW 2006, Australia

D. A. O'Brochta
Center for Agricultural Biotechnology,
University of Maryland Biotechnology Institute,
College Park, MD 20742, USA

Present address:

¹ Department of Biochemistry and Molecular Biology
University of California, Irvine, CA

² Department of Entomology
University of California, Riverside, CA 92521 USA

Introduction

As part of a continuing effort to produce germline transformants of non-drosophilid insects, we have adopted the approach of using plasmid-based assays to assess the mobility of transposable elements in the embryonic soma of a variety of insects. Two types of mobility assays have been developed: excision assays and transposition assays. Excision assays have been used as bioassays to detect the presence of endogenous forms of transposable elements. For example, we have previously reported the presence of *hobo*-like transposase activity in the housefly *Musca domestica* (family: Calliphoridae), using excision assays with the *hobo* transposable element from *Drosophila melanogaster* (Atkinson et al. 1993). We subsequently reported the isolation and characterisation of a functional *hobo*-like element, *Hermes*, from the *M. domestica* genome (Warren et al. 1994). *Hobo* transposition assays have demonstrated *hobo*'s ability to act as a gene vector in at least two non-drosophilid species (O'Brochta et al. 1994).

Recently, we reported the development of excision assays designed to measure the ability of the *D. mauritiana mariner* element to excise in insects (Coates et al. 1995). These assays demonstrated that the *mariner* transposable element from *D. mauritiana* is capable of accurate excision in the embryonic soma of two non-drosophilid insects: the Australian sheep blowfly *Lucilia cuprina* (family: Calliphoridae) and the Queensland fruit fly *Bactrocera tryoni* (family: Tephritidae). The majority of empty excision sites examined following *mariner* excision contained 3 bp from one of the *mariner* inverted terminal repeats, as is observed at empty excision sites in *D. mauritiana* chromosomes following *mariner* excision (Bryan et al. 1990). In both *L. cuprina* and *B. tryoni* there was an absolute requirement for the presence of a helper plasmid providing a source of exogenous *mariner* transposase.

This result was not unexpected given the evidence indicating the spread of *mariner* elements by horizontal

transmission. *Mariner*-like elements are widely distributed in a variety of species across a number of phyla and, in some cases, the phylogenies derived from the comparison of the DNA sequences of these *mariner* elements are different from the established phylogenies of their host species (Maruyama and Hartl 1991a,b; Capy et al. 1992; Garcia-Fernández et al. 1995; Robertson 1993; Lohe et al. 1995b; Robertson and Lampe 1995). The purified transposase from the horn fly *Haematobia irritans* has been demonstrated to be capable of mediating *mariner* transposition in vitro, suggesting that host-encoded factors are not necessary for *mariner* mobility (Lampe et al. 1996). This may, in part, provide an explanation for *mariner*'s apparent ability to cross species barriers.

The *mariner* element has previously been used as a gene vector to transform *D. melanogaster* and, more recently, *D. virilis* (Garza et al. 1991; Lidholm et al. 1993; Lohe et al. 1995a; Lohe and Hartl 1996). To determine the potential of the *mariner* gene vector system to produce germline transformants of non-drosophilid species, we developed a *mariner* transposition assay based, in principle, on the successful *hobo* transposition assay (O'Brochta et al. 1994). We present evidence that *mariner* is capable of accurate transposition in the embryonic soma of *D. melanogaster*, *L. cuprina* and *B. tryoni*. These results demonstrate that a gene vector system constructed from the *mariner* element should be capable of producing germline transformants of these and possibly other non-drosophilid insects. In addition, the reporter genes were cloned into different restriction sites of the *mariner* element, demonstrating the potential utility of the transposition assays for defining regions of the *mariner* element that are important for accurate transposition.

Materials and methods

Fly stocks

The strain of *D. melanogaster* used was *y; w67c23*. The *L. cuprina* strain was Standard Wild Type (SWT), a laboratory strain maintained at the CSIRO Division of Entomology, Canberra. The *B. tryoni* strain was a laboratory strain that had been maintained at the CSIRO Division of Entomology, Canberra, for approximately 55 generations.

Plasmid construction

All plasmids were constructed using standard molecular biological techniques as described in Sambrook et al. (1989). pKHsp82MOS is a helper plasmid containing a blunt-ended *SspI*-*HindIII* fragment from pBSMOS ligated into a blunt-ended *Bam*HI site of pKHsp82 (Coates et al. 1995, 1996). The *SspI* site is located 60 bp downstream from the 5' terminal repeat of *MOSI* and 110 bp upstream from the ATG start codon. The *HindIII* site delimits the 3' end of the *MosI* clone and includes 200 bp of flanking *D. simulans* genomic DNA. Two different donor plasmids were used that contained the *Escherichia coli* origin of replication (*ori*) and a kanamycin resistance gene (*kan*) within the *mariner* element. pBSMOSoriKan was constructed by inserting a 2.25 kb *PstI*

fragment from pKO (D. A. O'Brochta, unpublished), which contains *ori* and *kan*, into the *SalI* site of pBSMOS at nucleotide 350 (Medhora et al. 1988, 1991; Coates et al. 1995). pMOSS' + 3'oriKan was constructed by inserting the 2.25 kb *PstI* fragment into the *Bam*HI site of pBCMOS' + 3' (Coates et al. 1995). This effectively inserts the reporter genes into the *SacI* site of the *mariner* element at nucleotide 790. The chloramphenicol resistance gene was removed from plasmid sequences so that it did not compromise future selection of putative transposition products, creating pMOSS' + 3'oriKan. The target plasmid, pGDV1, bears a chloramphenicol resistance marker is derived from *Bacillus subtilis* and cannot replicate in *E. coli*.

Transposition assays

The assays (Fig. 1) are similar in principle to the *hobo* transposition assay (O'Brochta et al. 1994) in that they allow the identification and isolation of the products of inter-plasmid transpositions of a transposable element. The assays have been modified in two ways. Firstly, they measure the ability of a *mariner* element, rather than a *hAT* element, to transpose between plasmids. Secondly, the selection scheme used to detect transposition has been made more sensitive by using two selective markers. This assay will be described in more detail elsewhere (A. Sarkar et al. in preparation). Transposition of *mariner* results in the transfer of the kanamycin resistance gene and the *E. coli* origin of replication from the donor plasmid to the pGDV1 target plasmid. Transposition products can then be selected in *E. coli* as kanamycin- and chloramphenicol-resistant clones. The two different donor plasmids were used at a concentration of 0.25 µg/µl each, the helper plasmid at 0.5 µg/µl and the target plasmid at 1.0 µg/µl. Plasmids were micro-injected into pre-blastoderm embryos (Spradling and Rubin 1982), which were then allowed to develop in an oxygen-rich, humid environment for approximately 16 h at 18° C. The developed embryos were then heat shocked for 1 h at 37° C (42.5° C for *L. cuprina*) and allowed to recover at 18° C for 1 h. Plasmid DNA was recovered from an average of 60 embryos for each independent experiment (Hirt 1967) and introduced into DH10B cells (BRL) by electroporation; the cells were allowed to recover for 1 h at 37° C in 1 ml of SOC media (Sambrook et al. 1989), after which 5 µl was plated onto medium containing ampicillin (50 µg/ml) to determine the titre of donor plasmids recovered. The remaining cells were then pelleted and resuspended in 100 µl of LB media (Sambrook et al. 1989) and plated onto LB media containing chloramphenicol (10 µg/ml) and kanamycin (25 µg/ml).

Plasmid DNA was prepared from colonies surviving selection on medium containing chloramphenicol and kanamycin (Qiagen QIAprep Spin Plasmid Minipreps) and analysed by restriction enzyme digestion and electrophoresis. Clones that appeared to be products of transposition events were analysed by double-stranded DNA sequencing with internal *mariner* primers MOSTL (5'-TGGTGGTTCGACAGTCAAGG-3') and MOSTR (5'-ACAAA-TTGCCCAGAGATGG-3'). DNA sequencing was performed by the chain-termination method of Sanger et al. (1977), using the Superbase Sequencing Reagent Kit (Bresatec) and modified T7 DNA polymerase (Pharmacia) under conditions recommended by the suppliers. To avoid the possibility of plasmid contamination between experiments, pipette barrels were bleached before every plasmid rescue and aerosol-resistant pipette tips were always used.

Results

Transposition

Mariner transposition products were recovered, albeit at different frequencies, from all the species tested (Table 1). The recovery of transposition products was absolutely dependent upon the presence of the exogenous source of

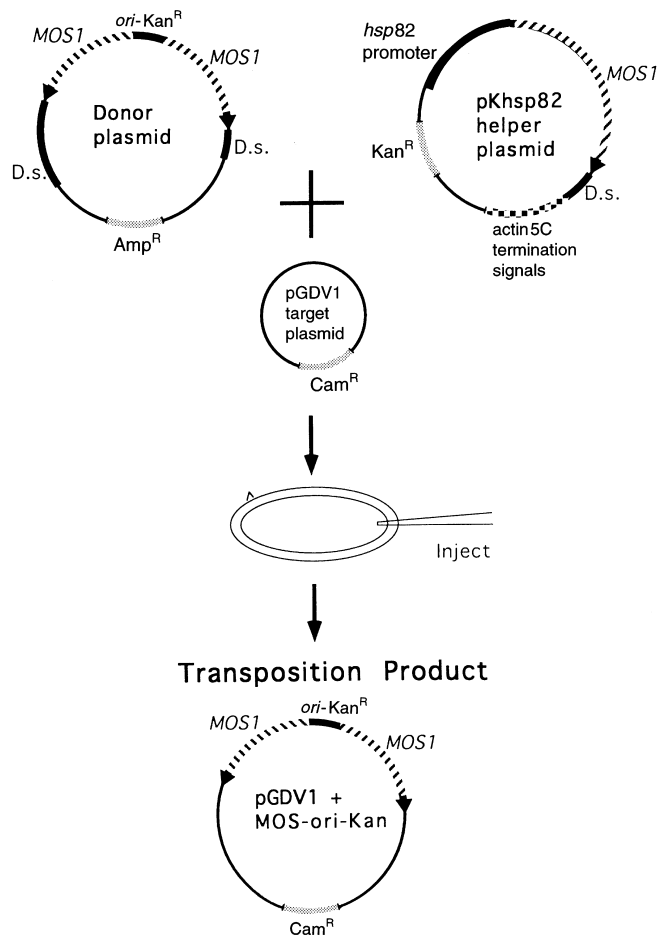


Fig. 1 *mariner* transposition assay. The donor, helper and target plasmids are injected into preblastoderm embryos. Approximately 16 h later the developing embryos are heat shocked and plasmids recovered and transformed into *Escherichia coli*. Kanamycin- and chloramphenicol-resistant plasmids, resulting from the transposition of *mariner* from the donor to the target plasmid, were selected. *Hatched areas* denote *Mos1* sequences, with inverted terminal repeats shown as *arrowheads*. The *solid areas* represent the flanking *D. simulans* genomic DNA. The position of the *hsp82* promoter and *Actin5C* polyadenylation signals are shown, as are the positions of the antibiotic resistance genes

mariner transposase provided by the hsp82MOS helper plasmid. Independent insertions into the pGDV1 sequence are shown in Fig. 2. The transposition assay plasmids were also introduced directly into *E. coli* DH10B cells without passage through insect embryos. After the screening of 2.86×10^6 donor plasmids, no chloramphenicol- and kanamycin-resistant colonies were recovered.

After the screening of 2.13×10^5 donor plasmids from *D. melanogaster*, 25 transposition products were recovered as determined by restriction enzyme digestion. Digestion of transposition products with *HincII* produces internal *mariner* fragments of constant size and variable sized flanking fragments, the sizes of which were dependent on the insertion site in pGDV1. Diagnostic internal fragments are obtained from the two different donor plasmids, which allows determination of the origin of the transposed *mariner* element. The DNA sequence of the junction points of *mariner* insertion into pGDV1 was determined. In each case the transposed sequences were delimited by the *mariner* terminal repeats. All the insertions had occurred at TA residues, and duplicated these.

When the transposition assay was performed in *L. cuprina* and *B. tryoni*, transposition products with the same structure as those from *D. melanogaster* were recovered from both species. This was determined from the *HincII* digestion pattern and the DNA sequence at the junction points. Some 1.84×10^5 donor plasmids were screened from *L. cuprina* and 7 transposition pro-

Table 1 Frequencies of transposition products recovered, with (+) and without (-) *mariner* transposase, from developing embryos

	<i>D. melanogaster</i>		<i>L. cuprina</i>		<i>B. tryoni</i>	
<i>mariner</i> transposase	-	+	-	+	-	+
Donor plasmids screened ($\times 10^5$)	1.22	2.13	5.73	1.84	12.4	10.52
Transpositions	0	25	0	7	0	9
Frequency (%)	-	0.012	-	0.004	-	0.0009

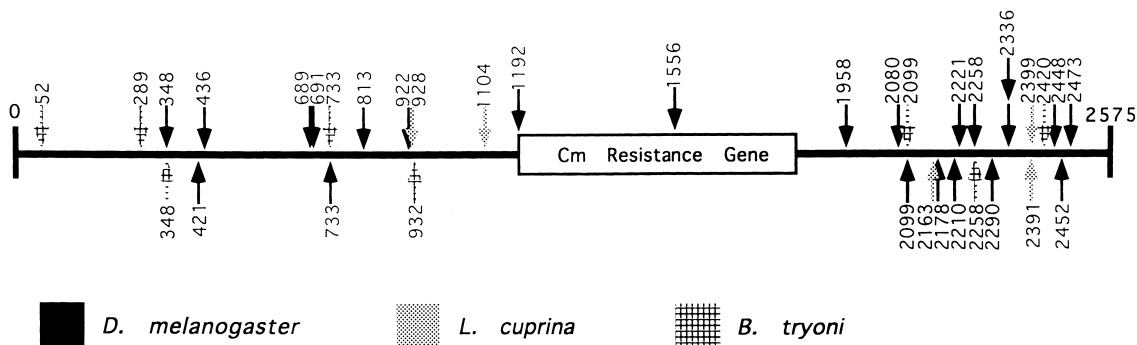


Fig. 2 Map of the pGDV1 target plasmid showing the positions of independent insertions of *mariner* recovered from *D. melanogaster* (filled arrows), *L. cuprina* (stippled arrows) and *B. tryoni* (hatched arrows). The chloramphenicol resistance gene of pGDV1 is indicated by the open box. Arrows above the bar, pointing down, refer to *mariner*

element insertions in which the 5' end of the transposase coding region is to the left and arrows below the bar, pointing up, refer to elements inserted in the opposite orientation. Numbers refer to the nucleotide position of the insertions within pGDV1

ducts were recovered. About 1.05×10^6 donor plasmids were screened from *B. tryoni* and 9 transposition products were recovered. As for the transposition products recovered from *D. melanogaster*, all insertions occurred at TA residues and duplicated these, while only sequences delimited by the *mariner* terminal repeats transposed. The majority of *mariner* elements had inserted at different points in pGDV1 in each species and between species. However, two independent insertions had occurred at position 2336 in *D. melanogaster* and four of the insertions in *B. tryoni* also occurred in *D. melanogaster* albeit in opposite orientation.

Donor plasmids

As described in the Materials and methods section, two different donor plasmids, each at the same concentration, were simultaneously used in the transposition assays. The two donor plasmids differ with respect to the locations of the *ori* and *kan* reporter genes within the *mariner* element. The *ori* and *kan* sequences have effectively been inserted into the *Sal*I (pBSMOSoriKan) and *Sac*I (pMOS5' + 3'oriKan) sites of the *Mariner* element at nucleotides 350 and 790, respectively. Of the 25 transposition products recovered from *D. melanogaster*, 15 of the sequences originated from pBSMOSoriKan and 10 from (pMOS5' + 3'oriKan). Two of the products recovered from *L. cuprina* originated from pBSMO-SoriKan with the remaining five products originating from pMOS5' + 3'oriKan. Five of the products recovered from *B. tryoni* originated from pBSMOSoriKan with the remaining four products originating from pMOS5' + 3'oriKan. Therefore there is no significant difference in the transposition frequencies of the two donor elements.

Discussion

The evidence for the spread of *mariner* elements by horizontal transmission in insect species suggests that *mariner* is capable of transposition in heterologous cellular environments. Here we report direct functional evidence to support that hypothesis. When *mariner* elements and a source of exogenous *mariner* transposase were introduced into the embryonic soma of developing *D. melanogaster*, *L. cuprina* and *B. tryoni* embryos, transposition products were recovered that were a result of the transposition of a *mariner* element from a donor plasmid to a separate target plasmid. No chloramphenicol- and kanamycin-resistant colonies were recovered after the direct introduction of the transposition assay plasmids into the *E. coli* cells, indicating that the transposition events were occurring in the insect cells.

Transposition products were identified by screening for restriction fragments diagnostic for a complete *mariner* element. In all cases, in all species, the *mariner* element had inserted into the target plasmid. All the in-

sertions had occurred at TA residues and duplicated these, and only sequences delimited by the *mariner* terminal repeats were transposed. The structures of the transposition products were identical to those observed following chromosomal transposition of the *mariner* element in *D. mauritiana* and *D. melanogaster* (Jacobson et al. 1986; Bryan et al. 1990; Lidholm et al. 1993).

Mariner transposition products were recovered approximately three-fold less frequently from *L. cuprina* than from *D. melanogaster*. It is unlikely that this is due to varying levels of *mariner* transposase, as we have previously demonstrated that the *hsp82* promoter, used to express the *mariner* transposase from the helper plasmid, results in similar levels of reporter gene expression in both species (Coates et al. 1996). It is possible that the observed differences in *mariner* transposition frequency are due to differences in species-specific cellular factors that interact with the *mariner* transposable element system.

Polymerase chain reaction (PCR) analysis of *L. cuprina* genomic DNA indicated an absence of endogenous *mariner*-like DNA sequences, assayed under conditions known to detect such elements (data not shown; Robertson 1993). *D. melanogaster* also lacks *mariner* sequences (Jacobson et al. 1986; Maruyama and Hartl 1991a). *B. tryoni*, however, has been shown to contain several thousand copies of a *mariner*-like element within its genome (Frommer et al. 1996; C.L. Turney et al. unpublished data). It is possible that these endogenous elements are causing a negative interaction with the introduced *mariner* elements or *mariner* transposase, resulting in the observed lower frequency of *mariner* transposition in this species compared to both *D. melanogaster* and *L. cuprina*. We also observed a lower rate of *mariner* excision in this species compared to both *D. melanogaster* and *L. cuprina* (Coates et al. 1995).

Examination of flanking sequences 20 bp either side of each insertion sites did not reveal a consensus sequence. This is unlike the distribution of transposition events involving the *hobo* and *Hermes* elements, which show hot spots of insertions into target plasmids and a consensus target sequence (O'Brochta et al. 1994; A. Sarkar et al. in preparation). In the *mariner* assays there were five sites that had undergone two independent insertions and repeated assays may show these to be preferred sites of integration. There was also no apparent preference for orientation of the *mariner* element insertions with respect to the pGDV1 sequence. One insertion occurred within the chloramphenicol resistance gene. *E. coli* clones containing this plasmid showed poor growth and a low plasmid copy number as a result of this insertion.

We determined the average AT richness for each nucleotide position 20 bp upstream and 20 bp downstream from the TA target sites. The overall AT richness for the entire target plasmid was 69% and, as shown in Fig. 3, the AT richness at the majority of the 40 positions analysed reflect this high value. However at nucleotides -12, -4 and +4 from the TA target site

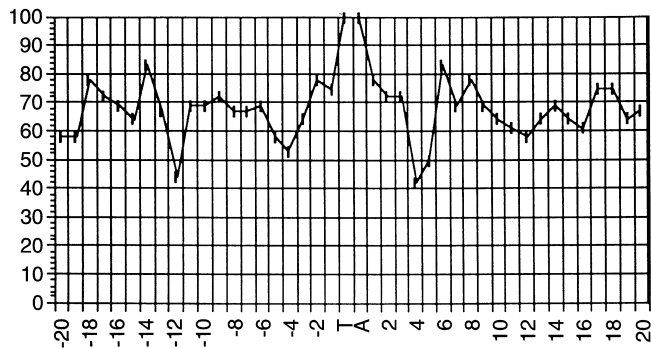


Fig. 3 The average frequency of AT base pairs (in percent) at nucleotide positions up to 20 bp upstream and 20 bp downstream from the TA insertion sites targeted by the *mariner* element in *D. melanogaster*, *L. cuprina* and *B. tryoni*

duplication there is a decrease in AT richness to 44%, 53% and 42%, respectively. While this pattern is by no means universal, it may reflect a preference for *mariner* elements to insert into TA residues which have GC nucleotides at these relative positions.

The placement of the reporter genes at different sites within the *mariner* element did not appear to effect the ability of the two donor elements to transpose. Lohe et al. (1995) found that *mariner* transformants of *D. melanogaster* marked with a *white*⁺ reporter gene at the *SacI* site were immobile in the soma, even in the presence of *mariner* transposase. This was in contrast to the frequent somatic movement of the *mariner peach* element in a *w^{pch}* strain of *D. melanogaster* (Lohe et al. 1995). The *peach* element had also been shown to excise and transpose at a high rate from the *w^{pch}* allele of *D. mauritiana* (Jacobson and Hartl 1985; Haymer and Marsh 1986; Jacobson et al. 1986; Bryan et al. 1987). It was postulated that this difference in mobility may be due to the insertion of the *white*⁺ marker gene within the element, or alternatively, that an important nucleotide sequence around the *SacI* site had been perturbed by this insertion. The data presented here discount these possibilities, although the donor element used here is smaller than the *mariner[white*⁺*]* transformation vector.

It is possible that the variation in *mariner* mobility is due to the difference in flanking genomic DNA present in the donor plasmids used in these experiments. The plasmids used in the transposition assays reported here, and the *mariner[white*⁺*]* transformation vector, all contained flanking *D. simulans* genomic DNA. It is possible that the secondary structure of these sequences allows higher rates of excision and transposition than the secondary structure of the *D. melanogaster* genomic DNA flanking the insertions of the *mariner[white*⁺*]* elements. Another example of apparent stability of *mariner* elements occurs in *D. sechellia* where two *mariner* elements are located in fixed positions within the genome, although in this case this could also be due to a lack of an active element (Capy et al. 1991).

The plasmid-based transposition assays provide a simple and rapid means to test these hypotheses. Donor

elements with different flanking genomic DNA or with reporter genes of various sizes inserted at different sites within the *mariner* element can be quickly constructed and assayed for mobility. The recent development of an in vitro transposition assay using a purified *mariner*-like transposase (Lampe et al. 1996), should enable *mariner* transposition assays to be performed using purified *mariner* transposase protein, and thus may assist in the in vitro analysis of the factors affecting *mariner* mobility.

Recently, the *Minos* element from *D. hydei* was used as a gene vector to genetically transform *D. melanogaster* and the Mediterranean fruit fly *Ceratitis capitata* (Loukeris et al. 1995a,b). The *Minos* element is a member of the *Tc1/mariner* superfamily and apparently shares *mariner*'s ability to function in heterologous cellular environments. Like *mariner* transposase, *Tc1* transposase has recently been demonstrated to function correctly in cell-free assays, suggesting that the transposases of the *Tc1/mariner* superfamily may not have a requirement for host-encoded factors (Vos et al. 1996). The use of excision and transposition assays with the *Minos* element would allow a rapid determination of the host range in which the *Minos* element can function.

Using plasmid-based transposition assays, we have shown that the *mariner* element is able to transpose in the embryonic soma of the blowfly, *L. cuprina*, and the tephritid, *B. tryoni*. This suggests that *mariner* will be able to form the basis of a germline transformation system for these two species. We plan to extend the use of the transposition assays to other non-drosophilid insects of medical and agricultural importance to determine the potential of *mariner* to transform these species. In addition, these assays will also enable a more thorough investigation into the effects of flanking DNA, construct size and insertion point on the rate of transposition of *mariner* elements.

Acknowledgements We would like to thank V. Baule and R. Russell for critically reviewing this manuscript, D. L. Hartl for the gift of the *Mos1* plasmid and D. Zeigler for the gift of the pGDV1 plasmid. C.L.T. is supported by an Australian Postgraduate Research Award. This work was also supported, in part, by grant GM48102 from the National Institutes of Health, USA.

References

- Atkinson PW, Warren WD, O'Brochta DA (1993) The *hobo* transposable element of *Drosophila* can be cross-mobilized in houseflies and excises like the *Ac* element of maize. *Proc Natl Acad Sci USA* 90:9693-9697
- Bryan GJ, Jacobson JW, Hartl DL (1987) Heritable somatic excision of a *Drosophila* transposon. *Science* 235:1636-1638
- Bryan G, Garza D, Hartl D (1990) Insertion and excision of the transposable element *mariner* in *Drosophila*. *Genetics* 125:103-114
- Capy P, Maruyama K, David JR, Hartl DL (1991) Insertion sites of the transposable element *Mariner* are fixed in the genome of *Drosophila sechellia*. *J Mol Evol* 33:450-456
- Capy P, David JR, Hartl DL (1992) Evolution of the transposable element *mariner* in the *Drosophila melanogaster* species group. *Genetica* 86:37-46

- Coates CJ, Turney CL, Frommer M, O'Brochta DA, Atkinson PW (1995) The transposable element *mariner* can excise in non-drosophilid insects. *Mol Gen Genet* 249:246–252
- Coates CJ, Howells AJ, O'Brochta DA, Atkinson PW (1996) The 5' regulatory region from the *Drosophila pseudoobscura hsp82* gene results in a high level of reporter gene expression in *Lucilia cuprina* embryos. *Gene*, 175:199–201
- Frommer M, Meats A, Sharkey D, Shearman D, Sved J, Turney C (1996) Sequences from eye colour genes, chorion gene and *mariner*-like transposable elements in the Queensland fruit fly *Bactrocera tryoni*. Proceedings of the 4th International Symposium on Fruit Flies of Economic Importance, in press
- García-Fernández J, Bayascas-Ramírez JR, Marfany G, Muñoz-Mármol AM, Casali A, Baguña J, Saló E (1995) High copy number of highly similar *mariner*-like transposons in planarian (Platyhelminthes): evidence for a trans-phyla horizontal transfer. *Mol Biol Evol* 12:421–431
- Garza D, Medhora M, Koga A, Hartl DL (1991) Introduction of the transposable element *mariner* into the germline of *Drosophila melanogaster*. *Genetics* 128:303–310
- Haymer DS, Marsh JL (1986) Germ line and somatic instability of a *white* mutation in *Drosophila mauritiana* due to a transposable genetic element. *Dev Genet* 6:281–291
- Hirt B (1967) Selective extraction of polyoma DNA from infected mouse cell cultures. *J Mol Biol* 26:365–369
- Jacobson JW, Hartl DL (1985) Coupled instability of two X-linked genes in *Drosophila mauritiana*: germinal and somatic mutability. *Genetics* 111:57–65
- Jacobson JW, Medhora MM, Hartl DL (1986) Molecular structure of a somatically unstable transposable element in *Drosophila*. *Proc Natl Acad Sci USA* 83:8684–8688
- Lampe DJ, Churchill MEA, Robertson HM (1996) A purified *mariner* transposase is sufficient to mediate transposition in vitro. *EMBO J*, 15:5470–5479
- Lidholm D-A, Lohe AR, Hartl DL (1993) The transposable element *mariner* mediates germline transformation in *Drosophila melanogaster*. *Genetics* 134:859–868
- Lohe AR, Lidholm D-A, Hartl DL (1995) Genotypic effects, Maternal effects and grand-maternal effects of immobilized derivatives of the transposable element *mariner*. *Genetics* 140:183–192
- Lohe AR, Moriyama EN, Lidholm D-A, Hartl DL (1995b) Horizontal transmission, vertical inactivation, and stochastic loss of *mariner*-like transposable elements. *Mol Biol Evol* 12:62–72
- Lohe AR, Hartl DL (1996) Germline transformation of *Drosophila virilis* with the transposable element *mariner*. *Genetics* 143:365–374
- Loukeris TG, Area B, Livadaras I, Dialektaki G, Savakis C (1995a) Introduction of the transposable element *Minos* into the germ line of *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 92:9485–9489
- Loukeris TG, Livadaras I, Arca B, Zabalou S, Savakis C (1995b) Gene transfer into the medfly, *Ceratitis capitata*, with a *Drosophila hydei* transposable element. *Science* 270:2002–2005
- Maruyama K, Hartl DL (1991a) Evolution of the transposable element *mariner* in *Drosophila* species. *Genetics* 128:319–329
- Maruyama K, Hartl DL (1991b) Evidence for interspecific transfer of the transposable element *mariner* between *Drosophila* and *Zaprionus*. *J Mol Evol* 33:514–524
- Medhora MM, MacPeck AH, Hartl DL (1988) Excision of the *Drosophila* transposable element *mariner*; identification and characterisation of the Mos factor. *EMBO J* 7:2185–2189
- Medhora M, Maruyama K, Hartl DL (1991) Molecular and functional analysis of the *mariner* mutator element Mos 1 in *Drosophila*. *Genetics* 128:311–318
- O'Brochta DA, Warren WD, Saville KJ, Atkinson PW (1994) Interplasmid transposition of *Drosophila hobo* elements in non-drosophilid insects. *Mol Gen Genet* 244:9–14
- Robertson HM (1993) The *mariner* transposable element is widespread in insects. *Nature* 362:241–245
- Robertson HM, Lampe DJ (1995) Recent horizontal transfer of a *mariner* element between Diptera and Neuroptera. *Mol Biol Evol* 12:850–862
- Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual (2nd edn) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York
- Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 74:5463–5467
- Spradling AC, Rubin GM (1982) Transposition of cloned P elements into *Drosophila* germ line chromosomes. *Science* 218:341–347
- Vos JC, De Baere I, Plasterk RHA (1996) Transposase is the only nematode protein required for in vitro transposition of *Tc1*. *Genes Dev* 10:755–761
- Warren WD, Atkinson PW, O'Brochta DA (1994) The *Hermes* transposable element from the housefly *Musca domestica* is a short inverted repeat-type element of the *hobo*, *Ac* and *Tam3* (*hAT*) element family. *Genet Res (Camb)* 64:87–97