Unexpected Stability of *mariner* Transgenes in Drosophila

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ABSTRACT

A number of *mariner* transformation vectors based on the *mauritiana* subfamily of transposable elements were introduced into the genome of *Drosophila melanogaster* and examined for their ability to be mobilized by the *mariner* transposase. Simple insertion vectors were constructed from single *mariner* elements into which exogenous DNA ranging in size from 1.3 to 4.5 kb had been inserted; composite vectors were constructed with partial or complete duplications of *mariner* flanking the exogenous DNA. All of the simple insertion vectors showed levels of somatic and germline excision that were at least 100-fold lower than the baseline level of uninterrupted *mariner* elements. Although composite vectors with inverted duplications were unable to be mobilized at detectable frequencies, vectors with large direct duplications of *mariner* could be mobilized. A vector consisting of two virtually complete elements flanking exogenous DNA yielded a frequency of somatic eye-color mosaicism of ~10% and a frequency of germline excision of 0.04%. These values are far smaller than those observed for uninterrupted elements. The results imply that efficient mobilization of *mariner in vivo* requires the presence and proper spacing of sequences internal to the element as well as the inverted repeats.

THE transposable element *mariner* is enumerated among a handful of sequences that have practical utility in serving as vectors for transformation in a variety of organisms (Hartl *et al.* 1997; Plasterk *et al.* 1999; Atkinson *et al.* 2001; Handler 2001; Hartl 2001). For *mariner*, the organisms successfully transformed so far include various bacteria, such as Escherichia and Mycobacterium (Rubin *et al.* 1999), Helicobacter (Guo and Mekalanos 2001), Vibrio (Chiang and Mekalanos 2000), and Neisseria (Pelicic *et al.* 2000). They also include various species of drosophilid insects (Lidholm *et al.* 1993; Lohe and Hartl 1996b; Horn *et al.* 2000), as well as an impressive list of nondrosophilids including mosquitoes (Coates *et al.* 1998; Moreira *et al.* 2000), silkworm (Wang *et al.* 2000), and housefly (Yoshiyama *et al.* 2000). The *mariner* element has proven successful in transforming eukaryotes as different as the protozoan Leishmania (Gueiros-Filho and Beverley 1997) and the vertebrates chicken (Sherman *et al.* 1998) and zebrafish (Fadool *et al.* 1998).

The wide variety of organisms that can be transformed by *mariner* vectors is not surprising in view of its extraordinary host range and ability to undergo horizontal transmission. Although the element was initially discovered in *Drosophila mauritiana* (Jacobson *et al.* 1986) and shortly thereafter in *Zaprionus tuberculatis* (Maruyama and Hartl 1991a) as well as *Hyalophora cecropia* (Lidholm *et al.* 1991), its broad taxonomic distribution began to become clear only with the work of Robertson (1993, 1995) and Robertson and MacLeod (1993) who found that >15% of ~400 insect species examined had one or more diverse subfamilies of *mariner* elements present in their genome. Soon thereafter *mariner* elements were found in centipedes and mites (Robertson and MacLeod 1993), nematodes (Sedensky *et al.* 1994), and planaria (Garcia-Fernàndez *et al.* 1993, 1995). *Mariner* elements have also been found in humans and other vertebrates (Auge-Gouillou *et al.* 1995; Morgan 1995; Oosumi *et al.* 1995; Robertson *et al.* 1996; Smit and Riggs 1996), as well as in plants (Jarvik and Lark 1998).

The canonical *mariner* element denoted *Mos1* is 1286 bp in length and includes 28-bp inverted repeats. The element contains an uninterrupted open reading frame encoding an active transposase of 345 amino acids (Medhora *et al.* 1988; Maruyama *et al.* 1991). The *mariner* element originally discovered, called *peach*, is also a full-length element. It differs from *Mos1* at 11 nucleotide sites. The *peach* element retains an open reading frame and is able to transpose, but the *peach* transposase differs from that of *Mos1* at 4 amino acid sites and is inactive (Maruyama *et al.* 1991; Medhora *et al.* 1991).

Both *peach* and *Mos1* have been used as transformation vectors in eukaryotes, with the active *Mos1* transposase used for mobilization into the target genome. The elements feature a number of convenient unique restriction sites, including *Sac*I at nucleotide position 787–792

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(numbering as in Jacobson *et al.* 1986), *Sal*I at position 349–354, *Sph*I at position 987–992, and *Cla*I at position 63–68 (this site is present in *Mos1* but not in *peach*).

In our original transformation experiments utilizing a *peach* vector with 11.9 kb of exogenous DNA inserted at the *Sac*I site (Lidholm *et al.* 1993), we observed that genomic insertions were remarkably stable in the presence of active transposase (Lohe *et al.* 1995). Genomic insertions of another vector with only 4.5 kb of exogenous DNA inserted at the *Sac*I site were also unexpectedly stable (Lohe *et al.* 1995). Both kinds of genomic insertions can be mobilized, but at low frequency (Lohe and Hartl 1996c).

To investigate whether *mariner* vectors with insertions of exogenous DNA at other unique cloning sites show levels of genetic stability comparable to insertions at the *Sac*I site, we carried out the experiments reported in this article. We studied vectors with insertions at the *Sal*I, *Sph*I, and *Cla*I sites. The inserted DNA was variously a *mini-white* marker (Thummel and Pirrotta 1992), a *yellow* marker (Patton *et al.* 1992), or an eye-specific enhanced green fluorescent marker responsive to the transcription factor Pax-6 (Berghammer *et al.* 1999; Horn *et al.* 2000). We also studied novel types of composite vectors in which the exogenous DNA was flanked by two essentially complete mariner elements in any of three possible orientations. In all cases with exogenous DNA inserted into the *Sal*I, *Sph*I, or *Cla*I site, we find that genomic insertions are largely refractory to mobilization by the active *Mos1* transposase. Somatic eye-color mosaicism is not observed, nor are progeny resulting from germline excision detected. Low levels of excision can, however, be detected by means of PCR amplification. With regard to the composite vectors, only genomic insertions with the flanking *mariner* elements in the same orientation (the "tail-to-head" configuration) were susceptible to mobilization by the *Mos1* transposase. We observed a rate of somatic mosaicism of ~1 in 10 and a rate of germline excision of ~1 in 2000. Although still substantially smaller than that rate of somatic mosaicism and germline excision observed with the *peach* element alone (100 and 14%, respectively), nevertheless the composite tail-to-head vector has a level of genomic instability far greater than any of the simple insertion vectors. These results are discussed in light of their implications for the use of *mariner* as a tool in genetic analysis and in the creation of transgenic organisms.

MATERIALS AND METHODS

Two types of transformation vectors were investigated. Simple insertion vectors consist of a single copy of *mariner* into which a marker gene was inserted at any one of a number of unique restriction sites. Composite vectors consist of a duplication of *mariner* sequence with a marker gene inserted at the junction of the duplicated regions.

Simple insertion vectors: Vectors were created with insertions in the mariner *Sal*I site (begins at nucleotide 349), the *Sph*I site (begins at nucleotide 987), or the *Cla*I site (begins at nucleotide 63). To prepare these vectors, the *mariner* element denoted *peach* was subcloned from the plasmid *pJJ1* (Jacobson *et al.* 1986) as an *Eco*RV-*Spe*I fragment and subcloned into *pBlue-ScriptII KS*, yielding the plasmid *pPch*. Polylinker sequence flanking the *Eco*RV-*Spe*I *peach* fragment was modified to remove the *Apa*I and *Sal*I sites as well as to introduce flanking *Eco*RI sites. Two adaptors were prepared, one by annealing oligonucleotides *Kpn/Hind-S* (5′-CGCGGCCGCGAATTCA-3′) with *Kpn/Hind-L* (5′-AGCTTGAATTCGCGGCCGCGGTAC-3′), and the other by annealing *Spe/Not-1* (5′-CTAGAATTCAGATCT-3′) with S*pe/Not-2* (5′-GGCCAGATCTGAATT-3′). These adaptors were ligated with the *Hin*dIII-*Spe*I *peach*-containing fragment of *pPch* and inserted into *pBlueScriptII KS* cleaved with *Kpn*I and *Not*I. The resulting derivative of *pPch* was designated *pPch-RR*.

The *hsp70:mini-white* cassette was subcloned as an *Xba*I-*Pst*I fragment from *PlwB* (Lidholm *et al.* 1993) into *pGem-3Zf(+)*. A trimolecular ligation was then used to create a duplication of polylinker at the ends of the *hsp70:mini-white* fragment by excising the *hsp70:mini-white* by digestion with *Bam*HI and *Sph*I and ligating with both an *Aat*II-*Sph*I fragment and a *Bam*HI-*Aat*II fragment from *pGem-3Zf(+)*. In the resulting plasmid, called *hsp/w-BSBS*, the *hsp70:mini-white* cassette is flanked by both *Bam*HI and *Sph*I sites. This *hsp70:mini-white* cassette was excised from *hsp/w-BSBS* with *Bam*HI and inserted into the *Sal*I site of the *peach* element in *pPch-RR* after half filling the cohesive ends with Klenow polymerase. This yielded the insertion vector designated *SalW*. (Both orientations of the *hsp70:mini-white* were recovered, but only one was studied further.) Similarly, the *hsp70:mini-white* cassette was excised from *hsp/w-BSBS* with *Sph*I and inserted into the *Sph*I site of the *peach* element in *pPch-RR*. This yielded the insertion vector designated *SphW*. (Again both orientations of the *hsp70:mini-white* were recovered, but only one was studied further.)

To obtain the insertion of *hsp70:mini-white* into the *Cla*I site of *mariner*, we had to modify the sequence of the *peach* element because, unlike the *Mos1* element, the *peach* element lacks a *Cla*I site at position 63. Accordingly, nucleotides 7–1280 of the peach element were excised from *pPch-RR* using the restriction enzyme *Bsr*GI, and this fragment was replaced with the corresponding fragment from the plasmid *pMos1* (Medhora *et al.* 1988). This operation completely converted the sequence of the *peach* element in *pPch-RR* into that of *Mos1*, yielding the plasmid *pMos-RR*. In the next step, the kanamycin-resistant element in the plasmid *pUC4K* was excised with *Bam*HI and inserted in the *Cla*I site of *pMos-RR* after rendering the cohesive ends blunt with Klenow fragment, which restores the *Bam*HI sites flanking the kanamycin-resistant insert. Replacing the kanamycin-resistance cartridge with the *hsp70:mini-white* using *Bam*HI restriction sites resulted in the vector designated *ClaW*. The vector *ClaY*, which carries a *yellow* marker gene in the *Cla*I site, was created from *pMos-RR* by excising a 5.2-kb *yellow*-containing *Sal*I fragment from *pCar-y* (Patton *et al.* 1992) and ligating it into the *Cla*I site of *pMos-RR* after filling the cohesive ends with Klenow fragment to produce blunt ends.

The vector designated *Sal*G contains a marker gene inserted in the *Sal*I site of the *Mos1* mariner element. The marker gene, 1.3 kb in length, consists of a coding sequence for an enhanced green fluorescent protein driven by an artificial promoter that is eye specific and responsive to the evolutionary conserved transcription factor Pax-6. The *SalG* vector is described in detail as *pMos{3xP3-EGFP}* in Horn *et al.* (2000).

Composite vectors: The vector designated *SemiComp* contains a direct duplication of the *Sal*I-*Sph*I fragment of *peach* with an *hsp:mini-white* inserted at the junction of the duplicated fragments. This vector was created from the simple insertion vectors *SalW* and *SphW*, using the *Apa*I restriction site in the *hsp:mini-white*. The 5′ part of *SphW* was isolated as a *Kpn*I-*Apa*I

fragment and used to replace the 5′ part of *SalW*. In the resulting vector *SemiComp* the *hsp:mini-white* sequence is flanked by a direct duplication of the *Sal*I-*Sph*I segment of the *peach* element.

Three types of composite vectors containing almost complete duplications of *peach* were also created. The first step in generating these vectors was the introduction of mutations in the inverted repeats destined to flank the marker gene in the composite vector. For this purpose a *peach* element was created in which a *Pst*I restriction site was introduced to replace the first nucleotide of the 5′ inverted repeat and the flanking TA duplication. This 5′-mutated element was amplified from the plasmid *pJJ1* (Jacobson *et al.* 1986), using the primers *5′-mut* (5′-CTGCAGGTGTACAAGTAGGGAATGT CGG-3′), which is specific for the 5′-terminal inverted repeat, and *3′-pch* (5′-TTGTAGCGTTACCTAGCGTC-3′), which is homologous to the sequence of the intron of the *white* gene adjacent to the 3′ end of the peach element in *pJJ1*. Similarly, the 3′-mutated element was amplified with the primers *3′-mut* (5′-CTGCAGGTGTACAAGTATGAAATGTCGT-3′) and *5′-pch* (5′-GTTTTGGCACAGCACTTTGTG-3′). Both mutated elements were cloned in the vector *pCRII* (Invitrogen, Carlsbad, CA). The resulting clones were stable only in the SURE strain of *Escherichia coli* (Stratagene, La Jolla, CA), which was used in all subsequent experiments. Before proceeding further, the integrity of the PCR products was verified by DNA sequencing.

The 5′-mutated and 3′-mutated peach elements were used to create three kinds of composite vectors, designated *TT* (tail-to-tail), *HH* (head-to-head), and *TH* (tail-to-head), in which the duplicated *peach* elements are present in the orientations 5′-3′/3′-5′ (*TT*), 3′-5′/5′-3′ (*HH*), and 5′-3′/5′-3′ (*TH*). In all cases the two innermost inverted repeats are the mutated ends and the outer inverted repeats are those of the unmutated *peach* element. In each type of vector an *hsp70:mini-white* gene was inserted between the innermost pair of inverted repeats. The *peach* inverted repeats directly adjacent to the *mini-white* gene are modified and not susceptible to excision by the *mariner* transposase (Lohe *et al.* 2000). The outermost inverted repeats are intact and could, in principle, be used by transposase to mobilize the entire vector.

To create the *TT* vector, the 3′-end mutated *peach* element was excised from *pCRII* with *Eco*RI and *Pst*I and the fragments were ligated in a reaction including a *Pst*I fragment containing *hsp70:mini-white* derived from *PlwB* (Lidholm *et al.* 1993) and plasmid *pSP72* (Promega, Madison, WI) that had been linearized with *Eco*RI. The *HH* configuration was created similarly, using the 5′-end mutated *peach* element from *pCRII*.

Construction of the *TH* vector made use of both the *Eco*RI-*Pst*I fragment containing the 5′ mutation and the *Eco*RI-*Pst*I fragment containing the 3′ mutation. These were ligated together with plasmid *pSP72* that had been linearized with *Eco*RI. The only *peach*-containing clones recovered were those containing the tandemly repeated elements in the tail-to-head configuration, yielding the plasmid *pSP-TH*. Then the *Pst*I fragment containing the *hsp70:mini-white* cassette from the plasmid *PlwB* (Lidholm *et al.* 1993) was inserted into the *Pst*I site of *pSP-HT*, creating the *TH* vector.

Insertion of the vectors into the genome: The original *SacW* vectors were introduced into the germline by *mariner*-mediated transposition using *Mos1* as a helper plasmid (Lidholm *et al.* 1993; Lohe *et al.* 1995). In the present analysis, the *SalG* vectors were also introduced into the germline via *mariner*-mediated transformation as described in Horn *et al.* (2000). The other vectors were introduced using the *Hermes* transformation system (O'Brochta *et al.* 1996) by injection into embryos of genotype w^{1118} or *y w*. Each of the vectors of interest is flanked by unique *Eco*RI sites in the plasmid in which they reside. The *Hermes* vectors were constructed from the plasmid *pBSHermes* w^{+} (O'Brochta *et al.* 1996) by removing the *white*-bearing *Eco*RI fragment in this plasmid and replacing it with an *Eco*RI fragment bearing the *mariner* vector and marker gene of interest. The resulting plasmid was employed in germline transformation using a helper plasmid *pBCHSHH1.9* (O'Brochta *et al.* 1996), which bears a *Hermes* transposase open reading frame under the control of a heat-shock promoter. Concentrations of plasmid and helper were both 0.5 μg/μl, and the methods were those described in Lozovskaya *et al.* (1996).

Excision assays: Genetic experiments to estimate the *in vivo* rates of somatic and germline excision of the inserted vectors were carried out as described in Lohe *et al.* (1995, 1997).

Molecular methods: The polymerase chain reaction (PCR) was used to detect excision products at the molecular level, using primers flanking the *Eco*RI insertion site in the *Hermes* vector, namely 5′-ATAAATGCTGTGCCTCTCTA-3′ and 5′-ATTGTTTGTAGTATTGC-3′. These primers amplify excision products to yield a PCR product of ~475 bp (51 bp of *Hermes* plus 42 bp of Drosophila sequence on the 5′ side of the 35-bp polylinker and 264 bp of *Hermes* plus 83 bp of Drosophila sequence on the 3′ side). The PCR conditions were 95° for 1 min, then 25 cycles of 95° for 20 sec, 50° for 90 sec plus 1 additional second for each cycle, and 72° for 90 sec plus 4 additional seconds for each cycle. The resulting PCR products were stored at 4°. The PCR was carried out in a PTC100 machine from MJ Research (Waltham, MA). Southern blotting and DNA sequencing were carried out as previously described (Nurminsky *et al.* 1998).

RESULTS

We examined a number of possible transformation vectors on the basis of the transposable element *mariner*. Some of these were simple insertion vectors having exogenous DNA inserted into unique restriction sites in the element, and others were composite vectors in which a large region (in most cases virtually all) of the *mariner* element was duplicated on either side of the exogenous DNA. The vectors differed in the nature of the exogenous DNA, which included a 4.5-kb *mini-white* gene, a 5.2-kb *yellow* gene, or a 1.3-kb *green fluorescent protein* (*GFP*) cassette. In some vectors the direction of transcription of the marker gene was in the same orientation as that of the *mariner* open reading frame and in other cases in the reverse orientation. Most of the vectors were introduced into the genome of *D. melanogaster* using the *Hermes* transformation system. At first we tried the *P*-element system, finding that many of the simple insertion vectors were genetically unstable when subcloned into standard *P*-element vectors, yielding deletions and rearrangements (data not shown). We also tried the *hobo* transformation system (Blackman *et al.* 1989) but found, as others have, that many of the standard laboratory strains produce low levels of functional *hobo* transposase, which compromised the assays for the excision of *mariner* vectors (data not shown). Finally we turned to the *Hermes* system (O'Brochta *et al.* 1996), which yielded genetically stable subclones, a high efficiency of transformation, and genetic stability in the germline of the laboratory strains we used for the analysis. In most cases we re-

covered more germline transformants than were actually analyzed for vector excision. About one-third of the *Hermes* transformants had multiple insertions as evidenced by Southern blots and/or *in situ* hybridization (data not shown). All of the results described below were obtained from transformants that carried only one *Hermes* insertion.

The vectors were tested for their ability to be excised by the functional *mariner* transposase produced by the immobile element *Mr182* as described in Lohe *et al.* (1995, 1997). *Mr182* is a *P[hsp70::Mos1, ry$^+$]-182* construct inserted in chromosome 2; the *hsp70::Mos1* sequence has a dual promoter in which the *heat shock 70 promoter (hsp70)* is fused to the *Mos1* promoter at nucleotide position 58–59 (Lohe *et al.* 1995). The dual promoter has high activity even in the absence of heat shock (Lohe *et al.* 1995). Rates of somatic excision and germline excision of *mariner* elements are highly correlated with each other and with the rate of transposition (Garza *et al.* 1991; Lohe *et al.* 1995, 1997; Lohe and Hartl 1996a, 1996c). The baseline rate of somatic excision of the *peach* element in the presence of *Mr182* is 100 percent—which is to say that every fly shows somatic mosaicism of its eye-color phenotype (Garza *et al.* 1991; Lohe *et al.* 1997, 2000). The baseline rate of germline excision of *peach* is 14.7% (Lohe *et al.* 2000); this value reflects the proportion of revertant chromosomes in the mosaic germline.

Simple insertion vectors: The structure of each of the insertion vectors is diagrammed in Figure 1, which shows the insertion site and orientation of the exogenous DNA. In all vectors except for *ClaW*, *ClaY*, and *SalG*, the *mariner* element is the *peach* element (Jacobson *et al.* 1986); the *mariner* element used for the vectors *ClaW*, *ClaY*, and *SalG* is *Mos1* (Garza *et al.* 1991). The *peach* and *Mos1* elements differ at 11 nucleotide sites, including one that creates the *Cla*I site in *Mos1*. The *Sac*I vector exists in two forms with either an 11.9-kb *white* insertion or a 4.5-kb *mini-white* insertion. Results with these vectors have been reported previously (Lidholm *et al.* 1993; Lohe and Hartl 1996c). Among the transformants, the level of somatic mosaicism is ~1%, which is lower than the baseline value by two orders of magnitude.

The *SalG* transformants could not be scored for somatic mosaicism because small nonfluorescent patches, if there were any, in an otherwise highly fluorescent eye would probably not be detectable. The rates of somatic excision for all of the simple insertion vectors in Table 1 are at least 400 times smaller than the baseline values for *peach*. Experiments with comparable numbers of progeny were carried out to estimate the rate of germline excision, but no germline excision was detected for any of the vectors. For seven independent *SalG* insertions, we examined progeny for nonfluorescent eyes resulting from germline excision and observed 0/831. Based on the binomial distribution, this value defines

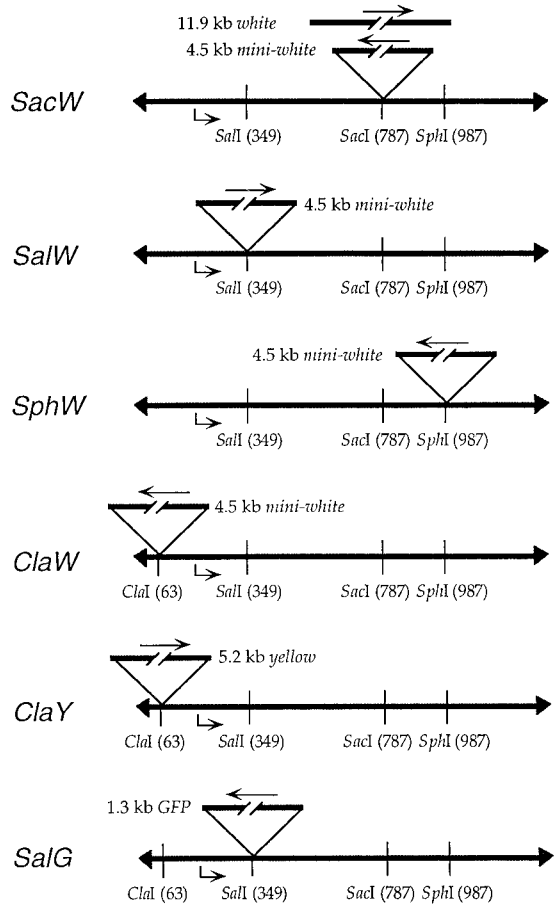

Figure 1.—Molecular structures of simple insertion vectors. Inserted exogenous DNA is not drawn to scale.

TABLE 1

Somatic excision of simple insertion vectors

Vector	Insertion site	Marker gene	No. of independently transformed lines analyzed	Total progeny examined	% progeny with somatic mosaicism
SalW	*Sal*I	*mini-white*	5	3443	0.23
SphW	*Sph*I	*mini-white*	3	2086	0
ClaW	*Cla*I	*mini-white*	2	1024	0
ClaY	*Cla*I	*yellow*	4	2961	0

an upper 95% confidence bound for the frequency of germline excision of the *SalG* vector of 0.36%, which is far smaller than the baseline value of 14.7% observed for the *peach* element alone (Lohe *et al.* 2000).

As Table 1 indicates, for most of the simple insertion vectors the rate of somatic excision is too low to be detected phenotypically as eye-color mosaicism among a few thousand progeny. Nevertheless, for some of the vectors, somatic excision can readily be detected at the molecular level in each individual fly. To detect the excision footprints we used PCR with primers flanking the *Eco*RI insertion site of the *mariner* vector in the *Hermes* transposon. Excision of a simple insertion vector would result in a predicted PCR product of 475 bp. A fragment of this size was prominent in the PCR product of each of 5–10 nonmosaic flies carrying either *SalW* or *SphW*, but not *ClaW* and *ClaY*. In the latter cases the excision product was virtually undetectable, either with amplification of DNA from single flies or from DNA isolated from homogenates of ~20 flies. A sample of 10–12 PCR products from apparent excisions of the other vectors was isolated and the relevant region sequenced to ascertain whether they exhibited the characteristic footprint of *mariner* excision. In each case the former location of the *peach* vector was now occupied by a sequence consisting of the TA duplication flanking *peach* plus one to three nucleotides from either the 5′ inverted repeat (5′-CCA-3′) or the 3′ inverted repeat (5′-TGA-3′). These sequences are typical of excision mediated by the *mariner* transposase (Bryan *et al.* 1990). (It should be noted that the PCR tests were not performed with the *SalG* transformants because this vector was introduced into the genome via *mariner*-mediated transformation rather than via *Hermes* transformation, and so the genomic sequences flanking the *SalG* transformants are unknown.)

Composite vectors: The low rate of excision of the simple insertion vectors might result from a tight size constraint whereby the *Mos1* transposase cannot efficiently mobilize elements substantially longer than the 1286-bp length of *peach* and *Mos1*. Alternatively (or perhaps in addition) *mariner* may contain two or more internal transposase-binding sites whose proper spacing is necessary for efficient mobilization. To examine this possibility, various types of composite transposons that contain duplications of some or all internal sequences were constructed from the *peach* element.

The structure of the composite vectors is shown in Figure 2. The *SemiComp* vector contains a direct duplication of the *Sal*I-*Sph*I region, between which the exogenous *mini-white* DNA is inserted. The fully composite vectors (*TT*, *HH*, and *TH*) have two almost complete *peach* elements flanking the exogenous *mini-white* DNA. These vectors differ in the orientation of the *peach* elements. In the *TT* vector, the 3′ ends of *peach* abut the exogenous DNA, and in the *HH* vector the 5′ ends of *peach* abut the exogenous DNA. In the *TH* configuration, both *peach* elements are in the same orientation,

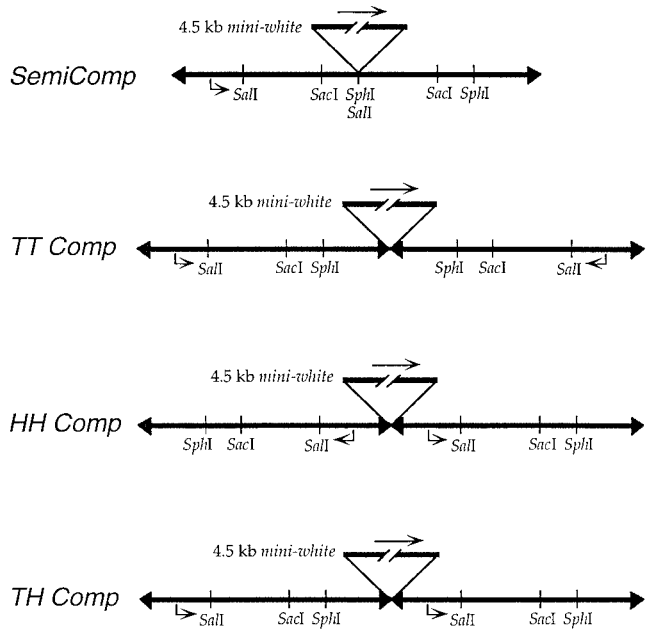

Figure 2.—Molecular structures of composite vectors. Inserted exogenous DNA is not drawn to scale.

with the 3′ end of the upstream element and the 5′ end of the downstream element abutting the exogenous DNA. In each of the fully composite vectors, the inverted repeat adjoined with the *mini-white* sequence has been mutated by deletion of the terminal nucleotide and the flanking TA, which have been shown to be critical for efficient recognition and cleavage by the *mariner* transposase (Lohe *et al.* 2000).

For transformants carrying each of the composite vectors, the frequency of somatic mosaicism is shown in Table 2. Although the two vectors with inverted duplications (*TT* and *HH*) show no evidence of somatic excision, the levels of somatic excision observed for the vectors with direct duplications (*SemiComp* and *TH*) are at least an order of magnitude greater than that of the simple insertion vectors (Table 1). Furthermore, the *TH* vector, which has all of the internal *peach* sequence duplicated, exhibits a significantly greater level of somatic excision than that of the *SemiComp* vector, which has only the *Sal*I-*Sph*I fragment duplicated ($P = 0.03$, Fisher's exact test). Although most of the eye-color mosaics carrying the *TH* construct have white (vector excision) patches on an otherwise red background, many of them also have one or more patches of eye facets that are exceptionally dark red. We attribute these to dosage effects of the *mini-white* marker resulting from somatic transposition of the *TH* vector, yielding cell lineages with two copies of the vector. In some crosses as many as 12.5% of the somatic mosaics showed such dark patches.

PCR analysis was carried out on flies carrying the *TT* and *HH* constructs, as well as on nonmosaic flies carrying the *TH* vector. For the *TT* and *HH* constructs, no

TABLE 2

Somatic excision of composite vectors

Vector	Structure	No. of independently transformed lines analyzed	Total progeny examined	% progeny with somatic mosaicism
SemiComp	*Sal*I-*Sph*I duplication	2	1693	8.4
TT	5′-3′/3′-5′	2	1203	0
HH	3′-5′/5′-3′	2	1341	0
TH	5′-3′/5′-3′	6	4376	10.2

The virgule (/) denotes the site of the *mini-white* insertion in the composite vectors that have virtually complete duplications of *peach*.

PCR product of the size predicted from vector excision was detected. This result implies that excision of the *TT* and *HH* constructs occurs at an extremely low rate, if it occurs at all, which is consistent with previous results that the 5′ inverted repeat and the 3′ inverted repeat are not equivalent or interchangeable with respect to transposase recognition and cleavage (Lohe *et al.* 2000). PCR products arising from apparent excision were readily detected in the *TH*-bearing flies, however, and as expected their sequences contained the characteristic footprints of excision mediated by the *Mos1* transposase. A sample of these footprints, each derived from a different animal, is shown in Table 3.

Although the frequency of somatic mosaics for the *TH* vector is 10-fold less than the baseline value for *peach*, it nevertheless suggested that the frequency of germline excision might be high enough to be detected in relatively small experiments. This proved to be the case. In crosses of males carrying an X-linked *TH* insertion with attached-X females, among 4515 male progeny we observed two revertants resulting from germline excisions. The estimated frequency of germline excision of the *TH* vector is thus 0.04%.

DISCUSSION

In this study we examined vectors with insertions of exogenous DNA into any one of several unique restriction sites (*Sal*I, *Sph*I, and *Cla*I) within the *mariner*, *peach*, or *Mos1* elements, motivated by previous findings that vectors with insertions into the *Sac*I site had a drastically impaired ability to be mobilized by the *Mos1* transposase (Lidholm *et al.* 1993; Lohe *et al.* 1995). We also examined composite vectors with direct or inverted duplications of part or virtually all of the peach element. The exogenous DNA consisted of a 4.5-kb *mini-white* sequence (*SalW*, *SphW*, *ClaW*, and all of the composite vectors), a 5.2-kb *yellow* sequence (*ClaY*), or a 1.3-kb sequence en-

TABLE 3

Excision footprints in PCR products from *TH* composite transformants

Flanking 5′ sequence	Excision footprint	Flanking 3′ sequence
GCACTTTGTGTTTAATTGATGGCGTA		TAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTAC		TAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTACC		TAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTACCA		TAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTACCA		TAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTACCA		TAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTACCA		TAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTA		ATAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTA		GATAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTA		TGATAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTA		TGATAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTA		TGATAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTA		TGATAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTA		TGATAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTA		TGATAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTA		TGATAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTA		TGATAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTA		TGATAAACCGCTTGGAGCTTCGT
GCACTTTGTGTTTAATTGATGGCGTA		TGATAAACCGCTTGGAGCTTCGT

coding GFP (*SalG*). The vectors were introduced into the genome of *D. melanogaster* either by *Mos1*-mediated transformation (*SalG*) or by *Hermes* transformation (all other vectors). Transformed lines containing single insertions were then tested for somatic and germline stability in the presence of the *Mos1* transposase source *Mr182*.

One important finding is that the simple insertion vectors have levels of somatic and germline excision that are at least 100-fold lower than the baseline level of uninterrupted *peach* elements. Low-level excision does take place, as shown by the analysis of PCR products from DNA of individual flies, which exhibited the characteristic sequence footprints of excision mediated by the mariner transposase. An exception should be noted for the *ClaW* and *ClaY* vectors, in which the expected PCR product was undetectable in standard gel assays.

Mobilization of some of the composite vectors was more efficient. Vectors containing a direct duplication of either the *Sal*I-*Sph*I fragment or almost the entirety of the element (except the 3′ nucleotide and the flanking TA duplication) were the most efficiently mobilized. The composite vector *TH* was mobilized with an efficiency significantly greater than that of the partial duplication. The frequency of excision of the *TH* vector, in which the flanking *peach* elements are almost completely intact, was 10-fold greater than that of any of the insertion vectors, although still 10-fold smaller than that of the peach element itself. The *TH* vector also yielded a frequency of germline excision of 0.04%, sufficiently high to be detected phenotypically in experiments of typical size. Mobilization of the fully composite vectors required that the two *peach* elements be in the same orientation. Those with the flanking *peach* elements in inverted orientation gave no evidence for excision that could be detected either phenotypically or by PCR amplification.

Size of vector *vs.* insertion site: The experiments suggest that overall size alone is not the primary cause of the impaired ability of the vectors to be mobilized. A simple insertion vector carrying 4.5 kb of exogenous DNA in the *Sac*I site is not detectably more prone to mobilization than is one carrying 11.9 kb, and a vector with 1.3 kb inserted into the *Sal*I site is not markedly more mobile than one carrying 4.5 kb in this site. The most efficiently mobilized composite vector we studied was the *TH* composite transposon, which has an overall size of 7.1 kb. This is 4.5 kb larger than the 2.66-kb *SalG* simple insertion vector. On the other hand, in the size range of 1.3- to 5.2-kb inserts that we have studied, while overall size is not the primary determinant of mobility, it may very well play some role in affecting the efficiency of mobilization. The *TH* composite exhibits a frequency of eye-color mosaicism of 10% and a germline excision rate of 0.04%. These values are still at least an order of magnitude smaller than the baseline values for *peach* and may to some extent reflect the 7.1-kb size of the vector in comparison with the 1.3-kb size of *peach* alone. In this context it should be noted that insect *mariner* elements found in natural populations that are closely related to *Mos1* are all very close to 1.3 kb in size (Capy *et al.* 1991; Maruyama and Hartl 1991b; Brunet *et al.* 2001).

Our results are most easily explained by supposing that efficient *mariner* mobilization requires the presence and correct spacing of certain internal sequences of the element relative to each other and to the inverted repeats. Insertion of exogenous DNA at almost any internal site would therefore impair the ability to be mobilized, although our data derive only from insertions at the *Cla*I, *Sal*I, *Sac*I, and *Sph*I sites. The finding that composite vectors can be mobilized ~10-fold more efficiently than simple insertion vectors is consistent with this interpretation. Some of the key internal sequences may be in the *Sal*I-*Sph*I fragment duplicated in the semicomposite vector, although the fully composite *TH* vector shows significantly greater mobilization, which may indicate that important internal sequences are also present outside the *Sal*I-*Sph*I region. The orientation of the internal sequences is evidently as important as their spacing, since we cannot detect mobilization of composite vectors that have their flanking *peach* elements in inverted orientation.

The hypothesis that essential internal sequences with the proper spacing and orientation are necessary for efficient mobilization is also consistent with recent results on the mobility of *peach* elements containing internal deletions. Analysis of 20 deletions revealed at least three internal regions whose integrity and spacing was necessary for efficient mobilization. These sequences are present within the regions bounded by nucleotides 229–586, 735–765, and 939–1066 (Lohe and Hartl 2001). The first of these regions contains the *Sal*I site and the third the *Sph*I site. We also found that mobilization was significantly impaired by a single nucleotide mutation at position 993 and by a double mutation at positions 161 and 179 (Lohe and Hartl 2001). How these internal sequences contribute to *mariner* mobilization is not known. Two obvious possibilities are that they are internal transposase-binding sites or that they are needed to form a secondary structure that is necessary for efficient recognition and cleavage. These models are of course not mutually exclusive.

***In vivo* results *vs. in vitro* results:** Our results on the impaired mobility of simple insertion vectors *in vivo* contrast with *in vitro* studies reporting that a *Mos1* vector consisting of nucleotides 1–64 and nucleotides 1253–1286 flanking a 1.1-kb *kanamycin-resistance* gene can transpose as efficiently as a complete *Mos1* element with the 1.1-kb *kanamycin-resistance* gene inserted into the *Sac*I site (Tosi and Beverley 2000). Two issues make the *in vitro* results difficult to compare with the *in vivo* results. The first is that the *in vitro* results are based on comparative data. Because the *mini-Mos1* vector was compared only with a *Mos1* vector having a *Sac*I insertion, it is unclear how the levels of transposition compare with those that would be found *in vitro* for *Mos1* alone. The second complication is that the *in vitro* experiments exploit the kanamy-

cin-selection system, so levels of transposition could be detected that are far smaller than can be observed phenotypically. Most of our simple insertion vectors do show levels of somatic excision that can readily be detected by PCR amplification, though not phenotypically, so perhaps the *in vitro* and the *in vivo* results are not as discordant as they first appear. On the other hand, there may be real differences in transposition efficiency for any of several reasons. For example, in typical *in vitro* systems, the concentration of transposase protein relative to vector DNA may be quite different from what it is *in vivo*. Furthermore, an *in vitro* system is an assay for transposition from purified DNA, whereas an *in vivo* system is an assay for transposition from chromatin.

Comparison with other transformation systems: Other systems for germline transformation based on insect transposable elements include the *P* element (Rubin and Spradling 1982), *hobo* (Blackman *et al.* 1989; Lozovskaya *et al.* 1996), *Hermes* (O'Brochta *et al.* 1996), *Tc*1 (Ivics *et al.* 1997), *Minos* (Loukeris *et al.* 1995), and *piggyBac* (Handler *et al.* 1998; Lobo *et al.* 1999). In these systems the transposase appears to be efficient at mobilizing vectors that contain exogenous DNA of 1–5 kb at any of a number of internal sites. In terms of delivery of exogenous DNA into the germline, the *Mos1* transformation system seems to be comparable to the others. The *Mos1* system has been used to transform protozoans (Gueiros-Filho and Beverley 1997), various species of insects (Lidholm *et al.* 1993; Lohe and Hartl 1996b; Coates *et al.* 1998; Horn *et al.* 2000; Moreira *et al.* 2000; Wang *et al.* 2000; Yoshiyama *et al.* 2000), and vertebrates (Fadool *et al.* 1998; Sherman *et al.* 1998). The efficiency of germline transformation is of the same order as that of other systems, even when the vectors are simple insertion vectors with exogenous DNA inserted at the *Cla*I site (Gueiros-Filho and Beverley 1997), the *Sal*I site (Horn *et al.* 2000), or the *Sac*I site (Lidholm *et al.* 1993; Lohe and Hartl 1996b). Indeed, Horn and Wimmer (2000) found that, when the *Sal*I-*Nru*I region of *Mos1* (nucleotides 350–950) was deleted and replaced with the 1.3-kb GFP marker, the efficiency of *Mos1*-mediated germline transformation was increased relative to that observed with the *SalG* vector.

On the other hand, the *Mos1* system manifests relative genetic stability of the vectors once they have been integrated into the germline. We are unaware of comparable findings published for other transformation systems. For *mariner*, the efficiency of mobilization of simple insertion vectors integrated into the genome of *D. melanogaster* is at least two orders of magnitude smaller than that of elements with no insert. The apparent discrepancy between relative efficiency of germline transformation compared with relative inefficiency of mobilization of the integrated vectors may relate to a difference between mobilization of elements that are present in molecules of plasmid DNA and those that are present in chromatin.

We do not know whether the relative stability of integrated simple insertion vectors is a characteristic of organisms other than *D. melanogaster*. It is possible that something peculiar about the chromatin of *D. melanogaster* does not occur in other organisms, which dramatically reduces the ability of simple insertion vectors to be mobilized once they have become integrated.

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LITERATURE CITED

Atkinson, P. W., A. C. Pinkerton and D. A. O'Brochta, 2001 Genetic transformation systems in insects. Annu. Rev. Entomol. **46:** 317–346.

Auge-Gouillou, C., Y. Bigot, N. Pollet, M. H. Hamelin, M. Meunierrotival *et al.*, 1995 Human and other mamm. genomes contain transposons of the *mariner* family. FEBS Lett. **368:** 541–546.

Berghammer, A. J., M. Klingler and E. A. Wimmer, 1999 Genetic techniques: a universal marker for transgenic insects. Nature **402:** 370–371.

Blackman, R. K., M. M. D. Koehler, R. Grimaila and W. M. Gelbart, 1989 Identification of a fully-functional *hobo* transposable element and its use for germ-line transformation of *Drosophila*. EMBO J. **8:** 211–217.

Brunet, F., T. Giraud, F. Godin and P. Capy, 2001 Do deletions of the *Mos1*-like elements occur randomly in the *Drosophilidae* family? J. Mol. Evol. (in press).

Bryan, G., D. Garza and D. L. Hartl, 1990 Insertion and excision of the transposable element mariner in Drosophila. Genetics **125:** 103–114.

Capy, P., K. Maruyama, J. R. David and D. L. Hartl, 1991 Insertion sites of the transposable element *mariner* are fixed in the genome of *Drosophila sechellia*. J. Mol. Evol. **33:** 450–456.

Chiang, S. L., and J. J. Mekalanos, 2000 Construction of a *Vibrio cholerae* vaccine candidate using transposon delivery and FLP recombinase-mediated excision. Infect. Immun. **68:** 6391–6397.

Coates, C. J., N. Jasinskiene, L. Miyashiro and A. A. James, 1998 *Mariner* transposition and transformation of the yellow fever mosquito *Aedes aegypti*. Proc. Natl. Acad. Sci. USA **95:** 3748–3751.

Fadool, J. M., D. L. Hartl and J. E. Dowling, 1998 Transposition of the *mariner* element from *Drosophila mauritiana* in zebrafish. Proc. Natl. Acad. Sci. USA **95:** 5182–5186.

Garcia-Fernàndez, J., G. Marfany, J. Baguñà and E. Saló, 1993 Infiltration of *mariner* elements. Nature **364:** 109.

Garcia-Fernàndez, J., J. R. Bayascas-Ramírez, G. Marfany, A. M. Muñoz-Mármol, A. Casali *et al.*, 1995 High copy number of highly similar mariner-like transposons in planarian (Platyhelminthe): evidence for a trans-phyla horizontal transfer. Mol. Biol. Evol. **12:** 421–431.

Garza, D., M. Medhora, A. Koga and D. L. Hartl, 1991 Introduction of the transposable element *mariner* into the germline of *Drosophila melanogaster*. Genetics **128:** 303–310.

Gueiros-Filho, F. J., and S. M. Beverley, 1997 Trans-kingdom transposition: mobilization of the *Drosophila* element *mariner* within the protozoan *Leishmania*. Science **276:** 1716–1719.

Guo, B. P., and J. J. Mekalanos, 2001 *Helicobacter pylori* mutagenesis by *mariner* in vitro transposition. FEMS Immunol. Med. Microbiol. **30:** 87–93.

Handler, A. M., 2001 A current perspective on insect gene transformation. Insect Biochem. Mol. Biol. **31:** 111–128.

Handler, A. M., S. D. Mccombs, M. J. Fraser and S. H. Saul, 1998 The lepidopteran transposon vector, piggybac, mediates germline transformation in the mediterranean fruit fly. Proc. Natl. Acad. Sci. USA **95:** 7520–7525.

Hartl, D. L., 2001 Discovery of the transposable element *mariner*. Genetics **157:** 471–476.

Hartl, D. L., A. R. Lohe and E. R. Lozovskaya, 1997 Modern thoughts on an ancyent *marinere*: function, evolution, regulation. Annu. Rev. Genet. **31:** 337–358.

Horn, C., and E. A. Wimmer, 2000 A versatile vector set for animal transgenesis. Dev. Genes Evol. **210:** 630–637.

Horn, C., B. Jaunich and E. A. Wimmer, 2000 Highly sensitive, fluorescent transformation marker for *Drosophila* transgenesis. Dev. Genes Evol. **210:** 623–629.

Ivics, Z., P. B. Hackett, R. H. Plasterk and Z. Izsvàk, 1997 Molecular reconstruction of *Sleeping Beauty,* a *Tc1*-like transposon from fish, and its transposition in human cells. Cell **91:** 501–510.

Jacobson, J. W., M. M. Medhora and D. L. Hartl, 1986 Molecular structure of a somatically unstable transposable element in Drosophila. Proc. Natl. Acad. Sci. USA **83:** 8684–8688.

Jarvik, T., and K. G. Lark, 1998 Characterization of *Soymar1*, a *mariner* element in soybean. Genetics **149:** 1569–1574.

Lidholm, D.-A., G. H. Gudmundsson and H. G. Boman, 1991 A highly repetitive, *mariner*-like element in the genome of *Hyalophora cecropia*. J. Biol. Chem. **266:** 11518–11521.

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

Lobo, N., X. Li and M. J. Fraser, 1999 Transposition of the *piggyBac* element in embryos of *Drosophila melanogaster, Aedes aegypti* and *Trichoplusia ni*. Mol. Gen. Genet. **261:** 803–810.

Lohe, A. R., and D. L. Hartl, 1996a Autoregulation of *mariner* transposase activity by overproduction and dominant-negative complementation. Mol. Biol. Evol. **13:** 549–555.

Lohe, A. R., and D. L. Hartl, 1996b Germline transformation of *Drosophila virilis* with the transposable element *mariner*. Genetics **143:** 365–374.

Lohe, A. R., and D. L. Hartl, 1996c Reduced germline mobility of a *mariner* vector containing exogenous DNA: Effect of size or site? Genetics **143:** 1299–1306.

Lohe, A. R., and D. L. Hartl, 2002 Efficient mobilization of *mariner in vivo* requires multiple internal sequences. Genetics **160:** 519–526.

Lohe, A. R., D.-A. Lidholm and D. L. Hartl, 1995 Genotypic effects, maternal effects and grand-maternal effects of immobilized derivatives of the transposable element *mariner*. Genetics **140:** 183–192.

Lohe, A. R., D. De Aguiar and D. L. Hartl, 1997 Mutations in the mariner transposase: the "D,D(35)E" consensus sequence is nonfunctional. Proc. Natl. Acad. Sci. USA **94:** 1293–1297.

Lohe, A. R., C. Timmons, I. Beerman, E. R. Lozovskaya and D. L. Hartl, 2000 Self-inflicted wounds, template-directed gap repair, and a recombination hotspot: effects of the *mariner* transposase. Genetics **154:** 647–656.

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

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

Maruyama, K., and D. L. Hartl, 1991a Evidence for interspecific transfer of the transposable element *mariner* between *Drosophila* and *Zaprionus*. J. Mol. Evol. **33:** 514–524.

Maruyama, K., and D. L. Hartl, 1991b Evolution of the transposable element *mariner* in Drosophila species. Genetics **128:** 319–329.

Maruyama, K., K. D. Schoor and D. L. Hartl, 1991 Identification of nucleotide substitutions necessary for trans-activation of *mariner* transposable elements in *Drosophila*: analysis of naturally occurring elements. Genetics **128:** 777–784.

Medhora, M., K. Maruyama and D. L. Hartl, 1991 Molecular and functional analysis of the *mariner* mutator element *Mos1* in Drosophila. Genetics **128:** 311–318.

Medhora, M. M., A. H. MacPeek and D. L. Hartl, 1988 Excision of the Drosophila transposable element *mariner*: identification and characterization of the Mos factor. EMBO J. **7:** 2185–2189.

Moreira, L. A., M. J. Edwards, F. Adhami, N. Jasinskiene, A. A. James *et al.*, 2000 Robust gut-specific gene expression in transgenic *Aedes aegypti* mosquitoes. Proc. Natl. Acad. Sci. USA **97:** 10895–10898.

Morgan, G. T., 1995 Identification in the human genome of mobile elements spread by DNA-mediated transposition. J. Mol. Biol. **254:** 1–5.

Nurminsky, D. I., E. V. Benevolenskaya, M. V. Nurminskaya, Y. Y. Shevelyov, D. L. Hartl *et al.*, 1998 Cytoplasmic dynein intermediate chain isoforms with different targeting properties created by tissue-specific alternative splicing. Mol. Cell. Biol. **18:** 6816–6825.

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

Oosumi, T., W. R. Belknap and B. Garlick, 1995 *Mariner* transposons in humans. Nature **378:** 672.

Patton, J. S., X. V. Gomes and P. K. Geyer, 1992 Position-independent germline transformation in *Drosophila* using a cuticle pigmentation gene as a selectable marker. Nucleic Acids Res. **20:** 5859–5860.

Pelicic, V., S. Morelle, D. Lampe and X. Nassif, 2000 Mutagenesis of *Neisseria meningitidis* by in vitro transposition of *Himar1*. J. Bacteriol. **182:** 5391–5539.

Plasterk, R. H. A., Z. Izsvak and Z. Ivics, 1999 Resident aliens: the Tc1/mariner superfamily of transposable elements. Trends Genet. **15:** 326–332.

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

Robertson, H. M., 1995 The Tc1-mariner superfamily of transposons in animals. J. Insect Physiol. **41:** 99–105.

Robertson, H. M., and E. G. MacLeod, 1993 Five major subfamilies of mariner transposable elements in insects, including the Mediterranean fruit fly, and related arthropods. Insect Mol. Biol. **2:** 125–139.

Robertson, H. M., K. L. Zumpano, A. R. Lohe and D. L. Hartl, 1996 Reconstructing the ancient *mariners* of humans. Nat. Genet. **12:** 360–361.

Rubin, E. J., B. J. Akerley, V. N. Novik, D. J. Lampe, R. N. Husson *et al.*, 1999 In vivo transposition of *mariner*-based elements in enteric bacteria and mycobacteria. Proc. Natl. Acad. Sci. USA **96:** 1645–1650.

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

Sedensky, M. M., S. J. Hudson, B. Everson and P. G. Morgan, 1994 Identification of a *mariner*-like repetitive sequence in *C. elegans*. Nucleic Acids Res. **22:** 1719–1723.

Sherman, A., A. Dawson, C. Mather, H. Gilhooley, Y. Li *et al.*, 1998 Transposition of the *Drosophila* element *mariner* into the chicken germ line. Nature Biotech. **16:** 1050–1053.

Smit, A. F. A., and A. D. Riggs, 1996 Tiggers and other DNA transposon fossils in the human genome. Proc. Natl. Acad. Sci. USA **93:** 1443–1448.

Thummel, C., and V. Pirrotta, 1992 New *pCaSpeR P*-element vectors. Dros. Inf. Serv. **71:** 150.

Tosi, L. R. O., and S. M. Beverley, 2000 Cis and trans factors affecting *Mos1 mariner* evolution and transposition in vitro, and its potential for functional genomics. Nucleic Acids Res. **28:** 784–790.

Wang, W., L. Swevers and K. Iatrou, 2000 *Mariner* (*Mos1*) transposase and genomic integration of foreign gene sequences in *Bombyx mori* cells. Insect Mol. Biol. **9:** 145–155.

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

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