The Transposable Element *mariner* Mediates Germline Transformation in *Drosophila melanogaster*

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**ABSTRACT**

A vector for germline transformation in *Drosophila melanogaster* was constructed using the transposable element *mariner*. The vector, denoted pMlwB, contains a *mariner* element disrupted by an insertion containing the wild-type white gene from *D. melanogaster*, the β-galactosidase gene from *Escherichia coli* and sequences that enable plasmid replication and selection in *E. coli*. The white gene is controlled by the promoter of the *D. melanogaster* gene for heat-shock protein 70, and the β-galactosidase gene is flanked upstream by the promoter of the transposable element *P* as well as that of *mariner*. The MlwB element was introduced into the germline of *D. melanogaster* by co-injection into embryos with an active *mariner* element, *Mos1*, which codes for a functional transposase and serves as a helper. Two independent germline insertions were isolated and characterized. The results show that the MlwB element inserted into the genome in a *mariner*-dependent manner with the terminal of the inverted repeats inserted at a TA dinucleotide. Both insertions exhibit an unexpected degree of germline and somatic stability, even in the presence of an active *mariner* element in the genetic background. These results demonstrate that the *mariner* transposable element, which is small (1286 bp) and relatively homogeneous in size among different copies, is nevertheless capable of promoting the insertion of the large (13.2 kb) MlwB element. Because of the widespread phylogenetic distribution of *mariner* among insects, these results suggest that *mariner* might provide a wide host-range transformation vector for insects.

The holy grail of insect transposable elements is an element with a broad host range able to serve as a vector for germline transformation in a wide variety of insect species. Numerous transposable elements present in insect genomes have been described, but few have been characterized in detail and evaluated as potential germline transformation vectors, except for a handful of elements found in *Drosophila melanogaster*. These include the transposable elements *P* (Engels 1989), *hobo* (Blackman and Gelbart 1989), *I* (Finnegan 1989) and *mariner* (Hartl 1989). The paradigm for germline transformation is the use of the *P* element to transform the germline of *D. melanogaster* (Rubin and Spradling 1982). In this case, the transformation vector consists of a *P* element with a large internal deletion within which is inserted the DNA sequence of interest. The defective *P* element containing the sequence of interest is introduced into the genome by transposition mediated by an active, "helper" *P* element co-injected into the embryonic germline. This transformation system has been widely used for identifying functional genetic units in *D. melanogaster*. One type of engineered *P* element—the enhancer trap—has also been widely used to identify genomic insertions near genes expressed only at particular times or in particular tissues (O'Kane and Gehring 1987; Bellen et al. 1989; Wilson et al. 1989). The enhancer trap contains the β-galactosidase gene from *Escherichia coli* under the control of the neutral *P*-element promoter, and so β-galactosidase is expressed only when the element transposes to a site in the genome near a strong enough enhancer. An analogous system of germline transformation has been developed based on the transposable element *hobo* (Blackman and Gelbart 1989), including a *hobo*-based enhancer trap (William Gelbart, personal communication).

The germline transformation systems for *Drosophila* currently in use were developed specifically for *D. melanogaster* using transposable elements originally discovered in the genome of this species. In the more general context of insect genetics, particularly with regard to insect pests, it would be important to develop a system that could be used to transform the germline in a wide variety of insect species. The host range of the *P* and *hobo* elements appears to be limited to *Drosophila* (Lansman et al. 1985; Daniels and Strausbaugh 1986; Daniels, Chovnick and Boussy 1990). Numerous attempts to transform species outside the genus *Drosophila* using the *P* element system have been unsuccessful (Engels 1989).

The transposable element *mariner* has an extraor-
dinarily broad host-range among insects. The mariner element was first described in Drosophila mauritiana (Jacobson, Medhoro and Hartl 1986) and a related element, 48% identical at the nucleotide level, was later found in the moth Hyalophora cecropia (Lidholm, Gudmundsson and Boman 1991). Using oligonucleotide primers designed to regions of amino acid conservation between the putative transposase genes in the mariner elements in these species, Robertson (1993) carried out assays with the polymerase chain reaction (PCR) among more than 75 insect species from several orders. He found mariner elements in 10 species representing six additional orders, and by phylogenetic analysis of the DNA sequences was able to determine that the mariner elements could be grouped into at least four major subfamilies. In a subsequent screen of over 400 species of insects and closely related arthropods, approximately 15% were positive for mariner by the PCR assay (Robertson 1993). Families of mariner elements, defined by significant sequence identity with the mariner element from D. mauritiana, also occur in some species far outside the insect-arthropod phyla, for example, in the nematode Caenorhabditis elegans (Philip Morgan, personal communication) and in the fungus Fusarium oxysporum (Pierre Capy, personal communication).

The widespread distribution of mariner and the close sequence similarity among some mariner elements from distantly related species suggest dissemination by horizontal transfer (Robertson 1993). The example analyzed in greatest detail concerns the mariner elements present in D. mauritiana and Zaprionus tuberculatus. Whereas the mariner elements in these species are 97% identical at the nucleotide level, the genes for alcohol dehydrogenase are only 82% identical (Maruyama and Hartl 1991a). Statistical analysis implies that the mariner elements in these species are almost certainly related by a horizontal transfer event considerably more recent than the time when the species separated from a common ancestor (Lawrence and Hartl 1992). If mariner can undergo horizontal transfer across a wide range of insect species, then this suggests that mariner might be developed as a versatile system for germline transformation.

On the other hand, most mariner elements are in a narrow range of lengths of approximately 1.3 kb (Maruyama and Hartl 1991a, 1991b), perhaps suggesting some size limitation on the ability of mariner elements to transpose. Furthermore, within groups of related species, the distribution of mariner is spotty—abundant in some species, absent in others (Jacobson, Medhoro and Hartl 1986; Maruyama and Hartl 1991a; Robertson 1993). For example, the genome of D. melanogaster appears to lack mariner elements, although it cannot be excluded that the species may contain divergent elements that have not been detected with the oligonucleotide primers currently in use. In any event, the discontinuous distribution may reflect the presence or absence of specific host factors needed for mariner integration or maintenance. Therefore, two major issues regarding mariner as the potential basis of a versatile germline transformation system are (1) can mariner integrate and be maintained in genomes in which it is normally not present? and (2) can mariner transposase mediate the transposition of transformation vectors substantially larger than itself? The first question has been answered in the affirmative because active mariner elements injected into embryos of D. melanogaster can integrate into the germline (Garza et al. 1991). In this paper we report that mariner can function as a transformation vector in D. melanogaster to introduce foreign DNA exceeding 13 kb in length. However, unlike the P-element system, mariner insertions carrying additional DNA appear to be unusually stable in both the germline and soma.

**MATERIALS AND METHODS**

**DNA manipulations:** General procedures for recombinant DNA manipulations are described in Sam brook, Frisch and Maniatis (1989). The strategy for the construction of the plasmid pMlwB (outlined in Figure 1) was to replace the P-element ends in the 12.5-kb enhancer-detector plasmid PlwB (Wilson et al. 1989) with sequences containing the inverted repeats of the autonomous mariner element Mos1 (Medhoro, MacPee and Hartl 1988). The terminal inverted repeats of mariner were isolated from a pBluescribe M13+ plasmid (Medhoro, Maruyama and Hartl 1991), which carries Mos1 flanked by 3.5 kb of Drosophila simulans DNA on the 5' side and 0.2 kb of D. simulans DNA on the 3' side. [The orientation and numbering of mariner sequences are as in Jacobson, Medhoro, and Hartl (1986).] The plasmid was digested with SacI, which has a single recognition site within the mariner transposon (at nucleotide position 787) but also at several sites in the flanking D. simulans sequences. The ends of the resulting fragments were made blunt with T4 DNA polymerase (thereby destroying the SacI sites) and a 1.3-kb fragment, extending from a position approximately 500 bp upstream of the 5' terminal repeat to nucleotide position 787 in Mos1, was purified in agarose and ligated into the Smal site of the Bluescript SK+ cloning vector (Stratagene) to yield the plasmid pA2L (Figure 1). Another fragment of 3.2 kb, extending from the same internal site in Mos1 to a position 2.7 kb downstream of the 3' terminal repeat, was digested with HindIII, which removes all but approximately 300 bp of the downstream sequences. The resulting 9.8-kb fragment was purified in agarose and ligated into the HindIII-HindIII sites of the pBluescript KS+ plasmid (Stratagene) to yield the plasmid pB2R (Figure 1).

The P-lac Z enhancer-detector plasmid PlwB (Wilson et al. 1989) was digested to completion with KpnI and then partially digested with HindIII to remove a 0.8-kb fragment that contains the terminal inverted repeats of the P element. The resulting 11.7-kb KpnI-HindIII fragment was purified in agarose and then joined in a triple ligation with a 1.3-kb HindIII-BamHI fragment from the plasmid pA2L, which contains the 5' portion of Mos1, and with a 0.8 kb BamHI-
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**Kpn1** fragment from the plasmid pB2R, which carries the 3’-portion of Mos1, yielding the plasmid pMlwB of 13.8 kb (Figure 1).

**Germline transformation:** Plasmid DNA was purified by CsCl-ethidium bromide equilibrium density-gradient centrifugation and used for injection of embryos. A DNA solution containing 450 µg/ml of pMlwB, along with 150 µg/ml of a pBluescribe M 13+ plasmid carrying Mos1, was injected into the pole plasm of *D. melanogaster* embryos of genotype *w*¹⁰⁸, essentially as described in Rubin and Spradling (1982).

**Southern blots and in situ hybridization:** Southern-transfer hybridization of genomic DNA (Southern 1975) was carried out essentially as described in Garza et al. (1991). Hybridization in situ to polytene chromosomes was carried out with probe DNA from the Drosophila white gene labeled with biotinylated 11-dUTP and detected with the horse-radish peroxidase system (Enzo), essentially as described in Lozovskaya, Petrov and Hartl (1993).

**Drosophila strains:** All strains described are *D. melanogaster*, and all crosses were carried out at 25°C on standard cornmeal-molasses medium. Descriptions of mutants and special chromosomes are found in Lindsley and Zimm (1992). Strain P735 carries the w¹⁰⁸ allele resulting from a mariner insertion in the X chromosome (Garza et al. 1991). The insertion M3-8, present in the strain w¹¹⁸; M3-8/CyO, is an autonomous Mos1 mariner element inserted into chromosome 2 (Garza et al. 1991). The Mos1 element in M3-8 was originally discovered in *D. mauritiana* (Bryan, Jacobsen and Hartl 1987), introgressed into *D. simulans* (Medhora, MacPeek and Hartl 1988), and cloned and introduced into the germline of *D. melanogaster* by P-element-mediated transformation (Garza et al. 1991). The w¹¹⁸ allele has a deletion of most of the white gene. The strains *y* w; *TM3/D* and *y* w; *CyO/Sco* were kindly provided by V. N. Jan.

**Plasmid rescue:** Genomic DNA from strains with putative MlwB insertions was prepared as described in Bender, Spierer and Hogness (1983). The DNA was digested with *PstI* or *SacI*, both of which lack recognition sites on the 3’-side of the polylinker adjacent to the Bluescript sequences in the MlwB element (Figure 1). Plasmid rescue was carried out as described in Wilson et al. (1989).

**DNA sequencing procedures:** Plasmids were purified by the alkaline lysis method (Sambrook, Fritsch and Maniatis 1989). DNA sequencing was carried out on an Applied Biosystems 373A Sequencer, using the Taq Dyedeoxy Terminator cycle-sequencing kit (Applied Biosystems) according to manufacturer’s specifications.

**RESULTS**

**General features of the plasmid pMlwB:** For consistency, we use the term pMlwB to refer to the plasmid in Figure 1. The MlwB element refers to sequences in the pMlwB plasmid between, and including, the outer termini of the mariner inverted repeats. The term MlwB insertion refers to the MlwB element incorporated into the Drosophila genome.

To avoid genetic instability of MlwB insertions in a
genetic background containing active P elements, the terminal repeats of P were completely removed from PlwB. However, all other sequences in PlwB were retained in the MlwB construct. Hence, inside the 5′ inverted repeat of MlwB, there is a P-lacZ fusion gene (O’Kane and Gehring 1987), in which the expression of the E. coli β-galactosidase gene is under the control of the weak constitutive transposase promoter of the P element. Approximately 700-bp upstream of the P promoter, pMlwB also contains the endogenous mariner promoter, which, although believed to be weak, could potentially effect the expression of the β-galactosidase gene. The upstream mariner sequences were retained because they might be required for germline transformation. The pMlwB plasmid also contains a white gene fused to the heat-shock protein 70 promoter (Klemenz, Weber and Gehring 1987) for use as a reporter gene. In addition, the MlwB element contains Bluescript KS− plasmid sequences, including an ampicillin-resistance gene and a bacterial origin of replication. The Bluescript KS− portion of MlwB is located at the 3′ end of the vector and carries a polylinker that may be used for direct cloning of adjacent genomic sequences by plasmid rescue (Pirrotta 1986). Because the 13.8-kb plasmid pMlwB (Figure 1) carries approximately 600 bp of D. simulans DNA between the mariner inverted repeats, the expected size of a mariner-mediated insertion of the MlwB element into the chromosome is 13.2 kb.

Isolation of MlwB transformants: Prior to the transformation experiments with MlwB, we first verified that the mariner element to be used as a helper in transformation was active. Accordingly, a pBluescribe M13+ plasmid carrying the active mariner element Mos1 (Medhora, MacPeek and Hartl 1988) was injected into the pole plasm of 240 D. melanogaster embryos of the P735 strain, which carries wFh. The wFh allele has an inactive mariner element inserted in the 5′ region of the untranslated leader upstream from the first exon (Bryant, Garza and Hartl 1990; Garza et al. 1991; Jacobson, Medhora and Hartl 1986). In the presence of active mariner, the inactive element in wFh undergoes excision at a very high frequency, resulting in somatic mosaicism manifested as pigmented patches in an otherwise peach-colored background (Bryant, Jacobson and Hartl 1987). Among 29 G0 survivors, 23 were fertile, of which one yielded heritable wach mosaicism indicative of Mos1 insertion. Hence, the mariner helper element is active and, in this experiment, had a germline transformation frequency of approximately 5%.

The pMlwB plasmid, along with the active mariner helper plasmid, were co-injected into the pole plasm of 1420 D. melanogaster embryos of genotype w1118. Among 301 surviving G0 offspring, 271 were fertile when backcrossed to w1118. Two of the fertile G0 flies yielded red-eyed transformants. These were designated M108, which produced one red-eyed progeny, and M159, which produced eight red-eyed progeny. Each G1 transformant was crossed with w1118, and stocks were produced by crossing red-eyed progeny males with females of the balancer strains y w; CyO/Seo and y w; TM3/D. In later generations both insertion lines were found to be homozygous viable.

In situ hybridizations, using white sequences as probe, are shown in Figure 2. The M108 insertion is in chromosome 3 at salivary position 64B16, and the M159 insertion is in chromosome 2 at 60D15-16. All eight G1 transformants from M159 carried an insertion at this same position.

Integrity of inserted DNA: If germline transformation in M108 and M159 was mediated by mariner, then (1) the insertions should include all DNA sequences between the extreme ends of mariner in pMlwB, and (2) the insertions should not include any of the D. simulans DNA present in pMlwB between the ends of mariner. These issues were addressed first with Southern hybridization analyses of genomic DNA from M108 and M159 (Figure 3). Genomic DNA from the M108 and M159 lines was digested with either SacI or PstI. The resulting genomic digests
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were separated by agarose electrophoresis, transferred to a membrane and hybridized with four probes covering different regions of pMlwB. Digestion with SacI is expected to yield five fragments from each genomic insertion, whereas digestion with PstI is expected to yield four fragments (Figure 1).

Probe A in Figure 3 consists of the HindIII-BamHI fragment of plasmid pA2L (Figure 1), which is specific for the 5' portion of MosI. If this region were intact in the MlwB insertions, SacI should generate a fragment of at least 3.4 kb, terminating at the SacI site in the lacZ gene and at the nearest SacI site in the flanking DNA; similarly, PstI should produce a fragment of at least 0.8 kb, terminating at the PstI site downstream of the 5' mariner sequences in MlwB and at the nearest PstI site in the flanking DNA. As Figure 3A indicates, both M108 and M159 yield bands that are much larger than the minimal sizes. If there were a major deletion in the 5' end of MlwB, at least one of the transformants might be expected to yield a fragment smaller than the minimal size. Furthermore, neither M108 nor M159 yielded a hybridizing 1.3-kb PstI fragment, generated from the PstI sites at 11 o'clock and 12 o'clock in Figure 1, which would be expected if the region of D. simulans DNA in pMlwB remained adjacent to the 5' end of mariner in the transformants. Based on these results, it appears likely that the 5' inverted repeat of mariner in MlwB constitutes the 5' boundary between the MlwB insertions and the flanking D. melanogaster genomic sequences in both M108 and M159.

Probe B in Figure 3 consists of the BamHI-Kpnl fragment of plasmid pB2R (Figure 1), which is specific for the 3' portion of MosI. In this case, the minimal size of both the SacI and PstI fragment is 3.1 kb, extending from the restriction sites in the BluescriptKS'-polylinker to the nearest SacI or PstI site in the flanking genomic DNA. Both M108 and M159 again yielded bands larger than the minimum size, suggesting that the 3' region of MlwB is present in both cases. If the junction between D. simulans DNA and the 3' inverted repeat of mariner present in pMlwB were retained in the insertions, a PstI fragment of 3.3 kb would be expected to hybridize with probe B (Figure 1). The absence of this fragment suggests that 3' inverted repeat in MlwB constitutes the 3' boundary between the MlwB insertions and the flanking D. melanogaster genomic sequences in both M108 and M159.

For analysis of sequences around hsp70-white, the filters were hybridized with probe C, consisting of the PstI fragment of pMlwB carrying the hsp70-white fusion gene (Figure 1). As expected from the restriction map of pMlwB, probe C hybridizes with two SacI fragments of 2.5 kb and 2.8 kb and with a PstI fragment of 4.5 kb, indicating that the hsp70-white gene is intact in both insertions. The additional bands in Figure 3C can be attributed to the endogenous hsp70 gene and to white sequences remaining in the w1118 allele.

Sequences around the lacZ gene were analyzed with probe D, consisting of the PstI fragment of pMlwB carrying the lacZ gene (Figure 1). This probe is expected to hybridize with internal SacI fragments of 1.7 kb and 2.5 kb in both transformants and with a third fragment extending from the SacI site in lacZ to the nearest SacI site in flanking genomic DNA. The 1.7-kb and 2.5-kb fragments are observed in both M108 and M159 (Figure 3D). The third fragment in M108 is 7 kb and in M159 is 10 kb, although the latter band is too weak to be evident in this exposure. Furthermore, both transformants contained the expected 4.5-kb PstI fragment from lacZ.

Consistent with the hypothesis that the MlwB insertions were mediated by mariner, the results in Figure 3 indicate that all major parts of the 13.2-kb region of pMlwB, extending from the 5' terminal repeat of mariner to the 3' terminal repeat, have been integrated in both the M108 and M159 transformants. This inference is subject to the limit of resolution of Southern blotting (approximately 50 bp in the size range of the DNA fragments in Figure 3) and therefore small deletions, particularly in the terminal sequences, may remain undetected. Additional experiments were therefore performed to investigate the integrity of the ends in greater detail.
For the 5' end, a more detailed Southern analysis was carried out on both M108 and M159 (Figure 4). Genomic DNA was digested with PsI in combination with either SalI or SspI, which have restriction sites in MosI at nucleotide positions 949 and 56, respectively (Medhora, Maruyama and Hartl 1991). Hybridization was carried out with the HindIII-BamHI fragment from plasmid p2A2L (Figure 1), which is specific for the 787-bp 5' fragment of MosI. The expected size of the PsI-SalI fragment is 446 bp and that of the PsI-SspI fragment is 741 bp. As can be seen in Figure 4, both M108 (lanes 1 and 2) and M159 (lanes 3 and 4) contain these fragments. Hence, at least the MosI sequences between nucleotide positions 56 and 787 are present in the MlwB insertions. Furthermore, the genetic instability of the MlwB insertions in the presence of active mariner (see below) suggests that the 5' terminal repeat is intact.

The integrity of the 3' ends of the MlwB insertions was investigated by direct DNA sequencing after plasmid rescue using the SacI and PsI sites adjacent to the Bluescript sequences (Figure 1). The nucleotide sequences of the 3' ends of the M108 and M159 insertions, as well the flanking genomic DNA, are shown in Figure 5. These data are consistent with insertion of MlwB by a mariner-dependent transposition: (1) both insertions had a 3' terminal inverted repeat identical to that in pMlwB, (2) the sequences of the 3' flanking DNA in both insertions were different from each other, as well as from the 3' flanking D. simulans DNA present in pMlwB, and (3) both insertions had the dinucleotide TA immediately flanking the 3' terminus of mariner.

**Co-integration of the mariner helper element:**
Two lines of evidence indicated that the active mariner element used as a helper had co-integrated with MlwB in the original M159 transformants. First, the mariner element contains single unique restriction sites for SalI, SspI and NheI; double digests of mariner DNA with SalI-NheI or SspI-NheI will, when probed with mariner, yield mariner-specific fragments of 894 bp and 1127 bp, respectively (Figure 6, lanes 7 and 8). These characteristic bands were observed in genomic DNA from M159 (Figure 6, lanes 3 and 4) but not in genomic DNA from M108 (Figure 6, lanes 1 and 2), suggesting that the mariner helper had co-integrated into the genome of the M159 strain along with MlwB. The larger fragments in Figure 6 are from the 3' end of MlwB.

The presence of MosI was also tested genetically by crossing M108 and M159 heterozygotes with the tester strain P735, which carries the w^ph^ allele in the X chromosome of D. melanogaster (Garza et al. 1991). The presence of active mariner in either M108 or M159 is expected to result in mosaic progeny in crosses with P735. This was the case with M159 but not with M108. It should be noted that somatic excision of the w^ph^ allele occurred at an unexpectedly low frequency (2/380 progeny from five single-male crosses as compared with virtually 100% mosaics in w^ph^, MosI controls). The low activity of the integrated mariner element may result from an altered nucleotide sequence or from position effects on expression at the particular site of insertion (Maruyama, Schoor and Hartl 1991). For experiments on somatic mosaicism in M159 (described below), the co-integrated mariner element was first eliminated from the strain by appropriate crosses and its absence confirmed with Southern blots.

**Somatic stability of MlwB:** Both M108 and M159 are genetically stable in the absence of active mariner elements. The genetic stability of the MlwB insertions in the presence of active mariner was tested by crossing females of M108 or M159 with males of genotype w^1118^; M3-8/CyO. If the transposase provided by the active mariner element in the M3-8 chromosome can function in trans to excise the MlwB insertion in somatic cells, somatic mosaicism, observed as unpigmented spots or regions in an otherwise red eye, would be expected in the F1 progeny. Somatic mosaicism was observed in some F1 progeny (Figure 7). However, as indicated in Table 1, the frequency of mosaicism was very low compared with controls carrying the w^ph^ allele, in which 100% of the w^ph^ progeny had somatic mosaicism in the presence of active mariner (data not shown). In addition, the mosaic sectors in the MlwB mosics were single patches and substantially larger than the typical salt-and-pepper pattern of mosaicism observed with w^ph^ (Figure 7A), suggesting that MlwB excision takes place earlier in eye development than with w^ph^.
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FIGURE 6.—Genomic blots indicating co-insertion of Mosl in M159. DNA samples were digested with SalI-Nhel (lanes 1, 3, 5 and 7) or SspI-Nhel (lanes 2, 4, 6 and 8) and the filter was probed with the BamHI-KpnI fragment from plasmid pB2R with the 3' part of Mosl. Arrows indicate the SalI-Nhel fragment (894 bp) and the SspI-Nhel fragment (1127 bp) of Mosl. Lanes 1, 2: M108; lanes 3, 4: M159; lanes 5, 6: w^{118}; lanes 7, 8: P755 (Mosl).

mariner sequences needed for recognition by Mosl are present in the MlwB insertions.

Germline stability of MlwB: The ability of Mosl to mobilize MlwB insertions in the germline was also examined on a scale sufficient to detect rates of transposition of 1% or greater. Crosses were carried out between M108 and M159 females and w^{118}; M3-8/+; M108/TM3, Sb and y w; +/-; TM3, Sb/D; approximately 2500 progeny were scored from each cross for the presence of TM3, Sb/D flies with wild-type eye color (indicative of transposition), but none were observed. In an equivalent P-element cross, performed as a control, using the P[ry^+ Δ2-3] (99B) element as a source of transposase (ROBERTSON et al. 1988), approximately 1% of the F2 progeny had undergone a germline transposition (data not shown).

DISCUSSION

We have shown that the small (~1.3 kb) transposable element mariner can be used successfully in transformation to introduce large pieces (13.2 kb) of DNA into the germline of D. melanogaster. Two independent transformants were obtained: one insertion in

FIGURE 7.—Eye-color mosaicism in the presence of Mosl. (A) Low-level mosaicism in w^{118}; (B) high-level mosaicism in w^{118}; (C and D) mosaicism in M108; (E and F) mosaicism in M159. Excision of the mariner element in w^{118} results in pigmented cells, whereas excision of MlwB results in unpigmented cells.
chromosome 2 (M159) and the other in chromosome 3 (M108). The insertions have the attributes expected of mariner-dependent insertions. The M159 transformants were recovered as a cluster of G1 offspring, which implies a premeiotic insertion also frequently observed with P-element transformation (Rubin and Spradling 1982). In the MlwB transformants, all sequences present in the pMlwB plasmid between the mariner-inverted repeats appear to be present and intact. Southern hybridizations show no evidence for deletion or rearrangement near the mariner-inverted repeats, and plasmid rescue and sequencing demonstrate that the genomic sequences flanking the mariner 3' inverted repeat differ from the D. simulans DNA that flanks the inverted repeats in pMlwB. It therefore appears that only sequences included within the termini of the mariner inverted repeats were transposed into the genome. Moreover, both insertions apparently occurred at a TA dinucleotide, which is characteristic of mariner (Bryan, Garza and Hartl 1990). In both insertions, eye-color mosaicism was observed in the presence of an active mariner element, which provides additional evidence that the inverted repeats in the MlwB insertions are intact.

Among 271 fertile G0 adults from embryos injected with MlwB, two yielded germline transformants, for an overall transformation frequency of 0.7%. Although the frequency appears relatively low in comparison with P-element transformation, experiments using large P-element vectors and helper elements capable of transposition on their own can yield comparable frequencies; for example, one 14.3-kb P-element vector containing white had a transformation frequency of 2% (Hazelrigg, Levis and Rubin 1984). There are a number of ways in which the efficiency of transformation in the mariner system might be increased. First, the observed frequency may be intrinsic to the use of Mos1 as the helper element and might be increased with different active mariner elements or elements whose transposase is under the control of a promoter that expresses at a high level in the germline. A second variable affecting transformation efficiency may be the size of the transformation vector; the MlwB element is 13.2 kb and, although its transformation into the germline is possible, smaller vectors may mobilize or integrate more efficiently. A third variable is the position of the inserted DNA in the transformation vector. Analysis of the P element has shown that sequences inside the inverted repeats can be critical in transposition (Mullins, Rio and Rubin 1989), and this may also be true of mariner. In MlwB, exogenous DNA is inserted into the SacI site at around nucleotide 787 (Jacobson, Medhora and Hartl 1986). Previous studies of mariner in D. teissieri have identified a deletion of 717 nucleotides that eliminates most of the 3' half of the element (Maruyama, Schoor and Hartl 1991). Approximately 80% of the copies of mariner in the Drosophila teissieri genome exhibit this deletion, which would suggest that the deleted elements retain all essential sequences needed for transposition. The 717-nucleotide deletion extends from nucleotides 544 through 1260. Because this includes the SacI site at nucleotide 787, the SacI site was chosen for insertion of exogenous DNA in constructing the MlwB element.

On the other hand, it is possible that the deleted elements in D. teissieri retain their ability to transpose only because of their small size or because of some feature of the novel junction created by the deletion, and efficient transposition may generally be inhibited by the presence of exogenous DNA at the SacI site. Finally, the efficiency of transformation may be affected by differences in the sequences of the inverted repeats in mariner. The Mos1 element and MlwB have four mismatches in the 28-bp inverted repeats (Medhora, Maruyama and Hartl 1991); this feature is shared with most mariner elements so far examined (Jacobson, Medhora and Hartl 1986; Maruyama and Hartl 1991b; Maruyama, Schoor and Hartl 1991; Capy et al. 1991, 1992). Nevertheless, these mismatches, although present in natural populations, may not maximize the efficiency of transposition.

The E. coli β-galactosidase was incorporated into the MlwB vector to provide enhancer-detector capability (O'Kane and Gehring 1987; Bell et al. 1989; Bier et al. 1989). Because both the M108 and M159 insertions are autosomal, the genetic procedures for detecting transpositions to new positions in the genome are not ideal or maximally powerful. Nevertheless, tests for transposition were carried out on a scale adequate enough to demonstrate that both the M108 and M159 insertions transposed with only low efficiency, if at all. This result is consistent with the relatively low level of eye-color mosaicism observed from somatic excision of the MlwB insertions in the presence of Mos1. The rate of transposition of MlwB must be affected by many of the same variables that determine the efficiency of transformation in the germline, and so the factors discussed above that may improve the efficiency of germline transformation may also increase the efficiency of transposition. On the other hand, there are also some important differences between germline transformation and transposition; for example, germline transformation mobi-

**TABLE 1**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mosaic progeny</th>
<th>Total progeny</th>
<th>Frequency (%)</th>
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<tbody>
<tr>
<td>M108</td>
<td>6</td>
<td>886</td>
<td>0.68</td>
</tr>
<tr>
<td>M159</td>
<td>14</td>
<td>1053</td>
<td>1.33</td>
</tr>
</tbody>
</table>

*Flies with mosaic eye color in either eye.*
lizes elements from plasmids whereas transposition mobilizes elements already in the genome.

Considerations about improvements in the efficiency of germline transformation should not obscure the principal finding that the mariner transposase is capable of supporting germline transformation of an MlwB element more than 10 times larger than the size of the mariner element itself. In view of the potential of mariner as a germline transformation vector in a variety of insects, it is also noteworthy that the germline transformation reported here was carried out in *D. melanogaster*, a species in which mariner is normally not present. The insertions of MlwB proved to be genetically quite stable, both in respect to somatic excision in the presence of active *Mos1* in the genetic background and in respect to transposition of MlwB to new positions in the genome. Although not ideal for purposes of genetic manipulation, the relative stability of the insertions may nevertheless be of considerable practical significance because stable transmittants may be necessary to implement some schemes of insect population regulation, such as those dependent on insertions that are conditional lethals or steriles.

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**LITERATURE CITED**


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