Insertion and Excision of the Transposable Element *mariner* in Drosophila

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ABSTRACT

The transposable element *mariner* is active in both germline and somatic cells of *Drosophila mauritiana*. Activity of the element is greatly enhanced in the presence of *Mos1*, a genetic factor identified as an autonomous copy of *mariner*. A strain of *D. mauritiana* containing *Mos1* and other copies of *mariner* was used to initiate a screen for visible mutations. More than 20 mutations were obtained, including alleles of *white*, *yellow* and *vermilion*. Six alleles were characterized at the molecular level, and all were found to contain a *mariner* element inserted into the affected gene. Four insertions into the *white* locus were sequenced to determine the exact site of insertion of *mariner*. There appears to be little sequence specificity requirement for *mariner* insertion, other than an absolute requirement for the dinucleotide TA, which is duplicated upon insertion. Sequences of phenotypically wild-type germline and somatic revertants obtained from various *white* alleles, including the previously isolated w^{pch} allele, were obtained using the polymerase chain reaction. *Mariner* excision is imprecise in both germline and soma, and the most frequent excision events are the same in the two tissues. Mutant derivatives of w^{pch} were also studied, and were found to exhibit a wide range of molecular structures and phenotypes.

TRANSPOSABLE elements are frequently used as a means of mutagenesis, and transposon tagging has been effective in cloning genes by the correlation of a mutant phenotype with the presence of a particular transposable element (BINGHAM, LEVIS and RUBIN 1981; SEARLES *et al.* 1982; FEDOROFF, FURTEK and NELSON 1984). Transposon insertions into particular genes, as well as secondary mutations such as deletions or rearrangements that are induced by excision of the transposon, can be used to analyze gene function (DANIELS *et al.* 1985; TSUBOTA and SCHEDL 1986; SEARLES *et al.* 1986; VOELKER *et al.* 1984). In *Drosophila*, the *P* element has been mobilized for mutagenesis and the cloning of genes such as *white* (BINGHAM, LEVIS and RUBIN 1981). The *P* transposon is also widely used as a method for transforming cloned DNA sequences back into the genome (RUBIN and SPRADLING 1982), and transformed genes located within a transposable DNA sequence can be mobilised to new genomic locations (SPRADLING and RUBIN 1982). More recently, the *Jumpstarter* system has enabled the construction of many strains each bearing a single *P* element insertion in a particular gene (COOLEY, KELLEY and SPRADLING 1988). Although the *P* element has been most widely used as a genetic tool in *Drosophila*, more recently the use of the *hobo* transposable element appears promising (YANNOPOULOS *et al.* 1987; BLACKMAN *et al.* 1987, 1989). In this paper we demonstrate genetic applications of the transposable element *mariner*. Among its convenient properties is the fact that it is active in somatic cells.

The transposable element *mariner* was first detected in an analysis of an unstable mutation in the *white* gene of *Drosophila mauritiana*, a sibling species of *Drosophila melanogaster* (JACOBSON and HARTL 1985; JACOBSON, MEDHORA and HARTL 1986). Strains of this species carry 20–30 copies of the 1.3-kb *mariner* element. Subsequently strains were isolated which showed enhanced rates of both somatic and germline excision of *mariner* from *white*, which were associated with a *trans*-acting factor designated *Mos1* (BRYAN, JACOBSON and HARTL 1987; BRYAN and HARTL 1988). The *Mos1* factor has been shown to be a particular copy of *mariner* capable of high rates of both excision and transposition (MEDHORA, MACPEEK and HARTL 1988). Strains carrying *Mos1* exhibit high mutation rates and give rise to new mutations during routine stock maintenance. It seemed likely that many of these might be induced by *mariner*. A study of new mutations induced by *mariner* in *Mos1* strains was undertaken to extend our understanding of both the *mariner* element and the affected genes.

In this paper we describe a mutation screen whereby the *Mos1* factor was used to mobilize *mariner* elements in the somatic mosaic strain E25H described by BRYAN, JACOBSON and HARTL (1987). We have studied six newly induced mutations of *yellow* and *white*

and have found that each is associated with an insertion of *mariner*. Each mutation shows somatic and germinal instability in the presence of *Mos1*, indicating that instability is a general feature of *mariner*. We conclude that *mariner* is capable of inducing mutations at appreciable frequencies, which implies that the transpositional activity of *mariner* is dysgenic within the germline. (We use the term *dysgenesis* in its general sense of "causing genetic damage.") Molecular analysis of *mariner*-induced *white* mutations and their derivative alleles defines the spectrum of *mariner*-induced mutational events. The results imply that there is little target specificity of *mariner* and that excision events are imprecise. The findings are discussed in terms of the molecular biology of the transposable element *mariner* and its utility as a mutagenic agent.

MATERIALS AND METHODS

Fly strains and crosses: The origin of the E25H strain of *D. mauritiana* has been described (BRYAN, JACOBSON and HARTL 1987). The strain carries the *X*-linked w^{pch} allele, which contains the transposable element *mariner* inserted in the 5′ untranslated region of *white* (JACOBSON, MEDHORA and HARTL 1986) as inferred from homology with the *D. melanogaster white* locus (PIROTTA and BROCKL 1984; O'HARE *et al.* 1984). The E25H strain also carries the dominant factor *Mos1* (formerly referred to as *Mos*), which promotes the excision of the *mariner* element inserted in the w^{pch} allele, giving rise to mosaics for eye color (BRYAN, JACOBSON and HARTL 1987; BRYAN and HARTL 1988). The strain is also germinally unstable for w^{pch}, generating phenotypically wild-type revertants at a frequency greater than 10^{-2}. Initially, the E25H strain, in common with other *D. mauritiana* strains tested, carried 20–30 copies of *mariner*. At the present time the strain carries approximately 45 copies of *mariner*, as determined by *in situ* hybridization (JONG-BONG KIM, personal communication). Flies were reared at 25° unless otherwise stated on standard cornmeal/agar/molasses Drosophila medium or Formula 4-24 instant medium (Carolina Biological, Burlington, North Carolina).

Mutation screen: A strain homozygous for the *Mos1* factor was made by sibmating single pairs of flies from the E25H strain for five generations. To permit the detection of novel alleles of *white*, a single phenotypically wildtype revertant male of w^{pch} was used to initiate the series of crosses in Figure 1. The resulting strain, designated E25HR1, was used for the mutation study. Approximately 50 individual sublines were maintained in order to ensure independence of mutational events. Following rapid expansion of E25HR1 the strain was screened for visible mutations for approximately 10 consecutive generations. Putative new mutations were placed individually in shell vials with siblings and homozygous strains were constructed by sib mating.

Generation of revertant alleles: Fly cultures initiated with single pairs of mosaic flies from various strains were screened for wild-type flies or flies with a different eye-color phenotype from the original mutant allele. Once a revertant had been obtained from a particular culture, the culture was discarded to ensure independence of reversion events. Revertant alleles were made homozygous prior to preparation of genomic DNA.

Polymerase chain reactions: The polymerase chain reaction (PCR) was performed essentially according to SAIKI *et al.* (1988). Where necessary to reamplify PCR products, DNAs were recovered from low melting temperature agarose gels prior to further PCR reactions. Single stranded DNA for sequencing was generated by the method of GYLLENSTEN and EHRLICH (1988). DNA sequencing of double-stranded PCR products was performed as described by DUBOSE and HARTL (1990).

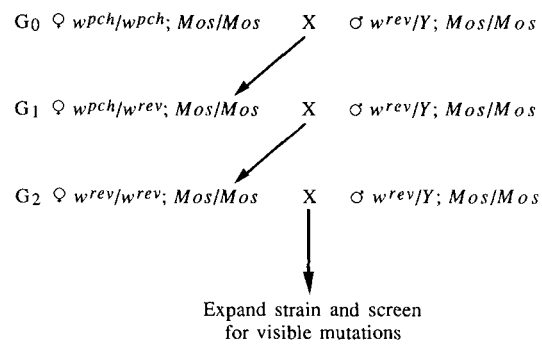

FIGURE 1.—Crosses used to generate phenotypically wild-type homozygous *Mos1/Mos1* strain for mutation screen. Prior to these crosses the strain had been single-pair mated for five generations. All crosses were single-pair matings. The male used to initiate the crosses was a phenotypically wild-type revertant of w^{pch} from the E25H strain. In the G_2 cross only approximately 50% of the females used were homozygous for the revertant allele. Vials still segregating for w^{pch} in the next generation were discarded.

Somatic excision: In order to study somatic excision of *mariner* from the w^{pch} allele, mosaic flies from the E25H strain and a mosaic strain of *D. simulans* were used to prepare DNA. Initially this was done with whole flies. However due to the fact that a mosaic fly contains many excision events it was decided to use single mosaic heads and to amplify the DNA with PCR. The use of *D. simulans* mosaic flies with the maternal-effect type of mosaicism (BRYAN and HARTL 1988) greatly reduced the number of excision events. Single heads were removed from flies and placed in 100 μl of PCR buffer (50 mM KCl, 10 mM Tris-Cl (pH 8.3), 2.5 mM $MgCl_2$, 0.1 mg/ml gelatin, 0.45% Nonidet P40, 0.45% Tween 20). This mixture was subjected to freeze-thaw conditions prior to the addition of 0.6 μl of 10 mg/ml proteinase K, and then incubated at 65° for 30 min. The proteinase K was inactivated by heating to 90° for 15 min. This crude extract was used to template DNA in PCR reactions. PCR products were separated in low melting point agarose and DNA fragments corresponding in size to the wild-type fragment were isolated. These fragments were reamplified by PCR prior to sequencing.

Southern blot analysis: *Drosophila* genomic DNA was prepared according to LIS, SIMON and SUTTON (1983). Restriction endonuclease digested DNAs were electrophoresed on 0.7% agarose gels and transferred to Hybond (Amersham) nylon membranes (SOUTHERN 1975). Filters were hybridized with probes labeled to high specific activity by random oligonucleotide primer extension (FEINBERG and VOGELSTEIN 1983).

Recombinant DNA techniques: Genomic DNAs for library construction were prepared by the method of KUNER *et al.* (1985). Phage libraries were constructed using λEMBL3 and λEMBL4. Genomic DNAs were partially digested with *Sau*3A and 15–20-kb fragments were ligated into the *Bam*HI site of the vectors. Recombinant phage were recovered by *in vitro* packaging with Gigapack (Stratagene Cloning Systems, San Diego, CA) and screened by the method of BENTON and DAVIS (1977). DNA fragments of interest were subcloned into M13mp18 and M13mp19 and were sequenced by the dideoxy chain termination method

Wildtype *white* sequence
-TTGATGGCGTAAACCGCTTGG-

w^{pch} sequence
-TTGATGGCGTAcca....mariner....tgaTAAACCGCTTG-

Sequence of w^{pchr1}
-TTGATGGCGTAtgaTAAACCGCTTG-

FIGURE 2.—Comparison of sequences of wild type, w^{pch}, and the wild-type revertant of w^{pch} used to initiate the mutation screen. The site of insertion of *mariner* in w^{pch} is at +3643 on the map of LEVIS, BINGHAM and RUBIN (1982). The sequence duplicated upon insertion of *mariner* into *white* is shown in bold face type. Sequences in lower case type and underlined represent *mariner* sequences.

of SANGER, NICKLEN and COULSON (1977) using Sequenase (United States Biochemical Corporation).

RESULTS

Molecular structure of *white* in strain E25HR1: Southern blots had previously shown that the *Bam*HI fragment containing *mariner* in w^{pch} had reverted to the wildtype size in w^{+} revertants, suggesting that the transposon had been deleted, though perhaps not precisely (BRYAN, JACOBSON and HARTL 1987). A phenotypically wildtype revertant of w^{pch}, designated w^{pchr1}, was used to establish the homozygous revertant stock designated E25HR1. The nucleotide sequences of the wildtype allele, the w^{pch} mutant, and the revertant allele in E25HR1 are summarised in Figure 2. The revertant allele actually arose from imprecise excision of *mariner* from w^{pch}. The two-base-pair duplication TA, characteristic of the insertion of *mariner* (JACOBSON, MEDHORA and HARTL 1986), remains in the revertant allele, flanking three additional base pairs matching the sequence at the 3′ end of the putative coding strand of *mariner* (*i.e.*, TGA).

Transposon tagging of visible mutations: Approximately 10^5 flies from E25HR1 were screened for visible mutations by expansion of the original stock. A total of 24 verified mutations were recovered, including alleles of *white* (4 alleles), *prune* (2 alleles), *yellow* (2 alleles), *vermilion* (2 alleles), *garnet*, and *lozenge*. These alleles were confirmed by complementation tests with previously identified alleles in *D. melanogaster*, or, where alleles were available, in *D. mauritiana* or *D. simulans*. Several of the alleles were observed to be genetically unstable, suggesting the possibility of transposon insertion. Furthermore, all four *white* and both *yellow* alleles showed somatic mosaicism and germinal instability in a *Mos1* genetic background, suggesting that *mariner* was inserted in these alleles.

Molecular analysis of *white* and *yellow* mutants: The *white* and *yellow* alleles were chosen for molecular study because their autonomous expression results in somatic mosaicism in the presence of *Mos1*. Southern blot analysis was carried out using DNA probes from *white* and *yellow*. These analyses detected insertion of novel DNA sequences approximately 1.3 kb in size into each of the mutant alleles. Restriction maps of the wild-type *D. mauritiana yellow* and *white* genes are given in Figure 3A.

The w^{pch} allele (Figure 3B(i), lane b), shows a 1.3-kb increase in size of a 3.0-kb *Bam*HI fragment, extending from coordinates +1.4 to +4.4 in the wild-type allele (lane a). One of the four new *white* alleles, w^{a49} (lane e), showed an insertion of 1.3 kb in the same *Bam*HI fragment as in the w^{pch} allele (lane b), while two other alleles, w^{a23} and w^{a41} (lanes c and d), show the same fragment size as wild type. It is also apparent that there is somatic excision of the insert from the w^{a49} allele (lane e), as indicated by the presence of the wild-type size fragment. The three alleles w^{a23}, w^{a41} and w^{a45} were found to contain approximately 1.3 kb of additional DNA inserted into the 2.9-kb *Bam*HI-*Eco*RI fragment, extending from approximately 0 to −3 on the restriction map (data not shown).

DNA blot analysis of the two *yellow* alleles, y^{a25} and y^{a45}, showed that they contained inserts of roughly 1.3 kb into a 2.6-kb *Eco*RI fragment (probe d), which corresponds approximately to the large intron in the *yellow* gene. (Figure 3B(ii), lanes f and g; the wild-type allele is shown in lane e.) A phenotypic revertant of allele y^{a25} shows the wild-type fragment size (lane h). A *Bam*HI-*Sal*I digest of DNAs from the same alleles shows that the 1.3-kb DNA fragment inserted into the mutant alleles contains a single *Sal*I site (Figure 3B(ii), lanes a–d). DNA from the y^{a25} allele, when digested with *Bam*HI and *Sal*I (lane b), shows 5.0- and 3.0-kb fragments (representing the 6.7-kb fragment with the insert) as well as the 6.7-kb wild-type fragment representing somatic excision of the insert. Similarly the y^{a45} allele (lane c) shows two fragments (4.4 kb and 3.7 kb), representing the 6.7-kb fragment with the insert, in addition to the 6.7-kb wild-type fragment resulting from somatic excision. All *mariner* elements cloned and sequenced to date contain a single *Sal*I site 349 bp from the 5′ terminus. Both mutant strains (lanes b, c, f and g) also show a band of the wild-type size (*i.e.*, 6.7 kb and 2.6 kb), which apparently results from somatic excision of the insertion in the presence of *Mos1*. The presence of 1.3-kb insertions containing a *Sal*I site, and the observation of instability in the presence of *Mos1*, are consistent with the hypothesis that the mutant phenotypes of the *white* and *yellow* alleles result from a *mariner* element inserted into or near the genes.

In order to determine the exact sites of insertion into *white*, genomic libraries were constructed using phage λ vectors λEMBL3 and λEMBL4 from strains carrying the mutant alleles of *white*. Clones containing DNA from the *white* locus were isolated, and fragments containing the insertions were subcloned into

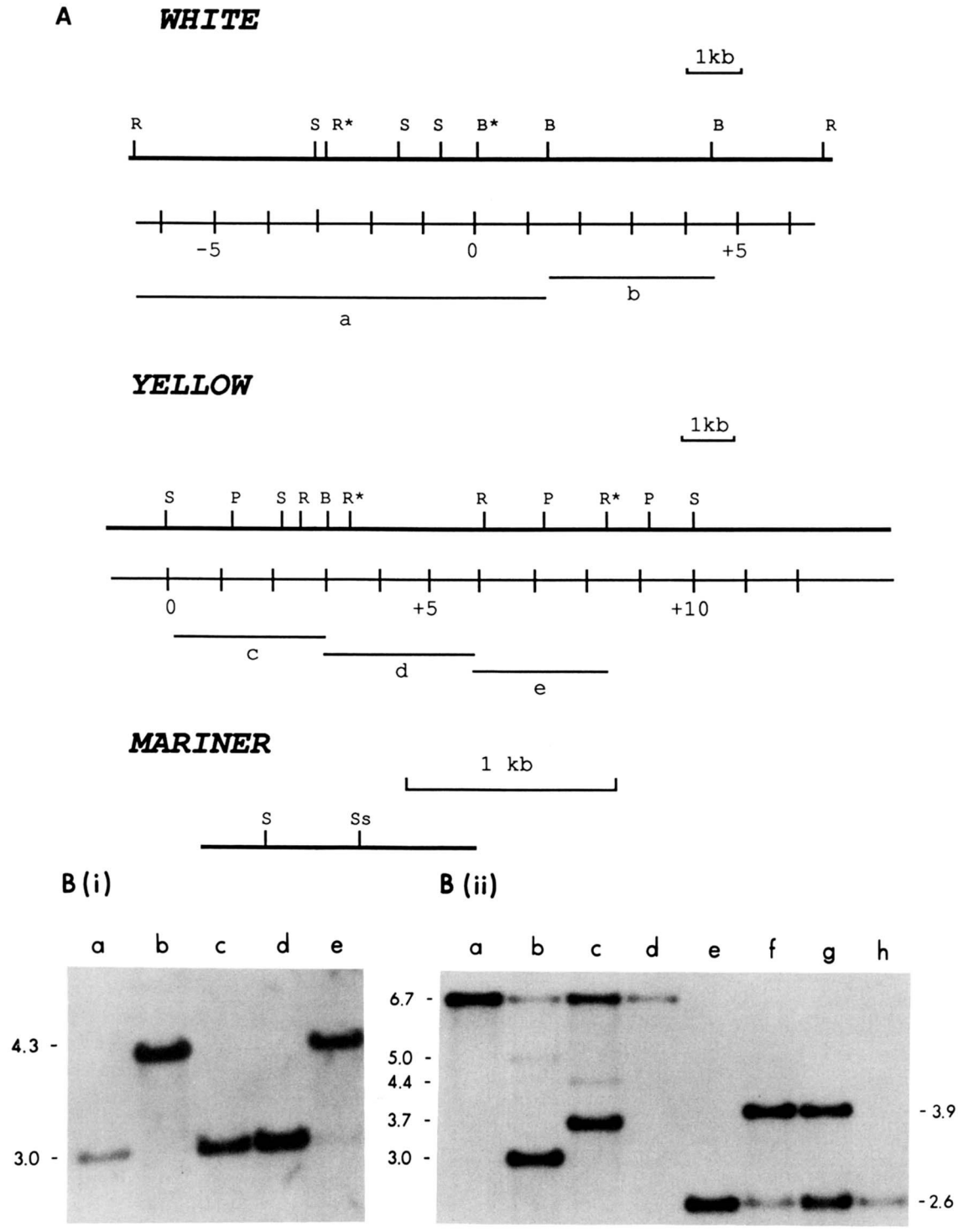

FIGURE 3.—Restriction maps of *white*, *yellow*, and *mariner*, and Southern blot analysis of *white* and *yellow* alleles derived from the mutation screen. (A) Restriction maps of *white*, *yellow*, and *mariner* in *D. mauritiana*. An asterisk denotes a restriction site not present in *D. melanogaster*. Coordinates are those of LEVIS, BINGHAM and RUBIN (1982) for *white* and CHIA *et al.* (1986) for *yellow*. DNA probes used for Southern blots and for screening genomic libraries are shown as horizontal lines (a–e). Probes c–e were provided by V. CORCES. Enzymes used were as follows: R (*Eco*RI), S (*Sal*I), B (*Bam*HI), P (*Pst*I), Ss (*Sst*I). (B) Southern blot analysis of *yellow* and *white* alleles. (i) *White* mutants. DNAs were digested with *Bam*HI and probed with a 3.0-kb *Bam*HI fragment (probe b). Lanes are as follows: a. wild type, b. w^{pch}, c. w^{a23}, d. w^{a41}, e. w^{a49}. (ii) *Yellow* mutants. DNAs were digested with *Bam*HI and *Sal*I (lanes a–d), and with *Bam*HI and *Eco*RI (lanes e–h), and probed with a 3.0-kb *Bam*HI-*Eco*RI fragment (probe d). Lanes are as follows: a and e, wild type; b and f, y^{a25}; c and g, y^{a45}; d and h, y^{a25r1}.

TABLE 1

Sequences flanking *mariner* insertions in *D. mauritiana*

Allele	Flanking sequence		Site[a]	Region
	5′....*mariner*....3′			
w^{pch}	TAATTGATGGCGTA	TAAACCGCTTGGAG	+3643	5′ leader
w^{a49}	GCACTTCGTGTTTA	TAATTGATGGCGTA	+3631	5′ leader
w^{a45}	CTATACTATTTGTA	TAACCCAGTTTGCG	−24	Intron 2
w^{a23}	GTTTGTTAAGGGTA	TATCTTGCATTACA	−712	Intron 3
w^{a41}	GGTATCTTGCATTA	TACATCTCAACTCC	−722	Exon 4?
w^{pchd7}	GGTTCGAACATATA	TAGATGTCTCGCAA		

[a] Signifies position of the *mariner* insert in *white* according to the coordinate system of LEVIS, BINGHAM and RUBIN (1982). Flanking sequences are given such that the leftmost sequence is that flanking the 5′ terminus of *mariner*. The w^{pchd7} allele is due to *mariner* inserting into the element at w^{pch}, 38 nucleotides from its 5′ end. The sequence underlined in w^{a41} corresponds to the intron acceptor site in *D. melanogaster*.

M13. The sequences flanking the insertions into *white* were determined using oligonucleotide primers homologous to sequences near the ends of *mariner*. Once the insertion sites were known, primers to *white* sequences were used to obtain the DNA sequences of the opposite strands. The sequences flanking the insertion sites were determined as well as approximately 100 bp of the inserted DNA. Sequences immediately flanking the known *mariner* insertions into *white* are given in Table 1. In all cases the sequence of the inserted DNA corresponded to that of a *mariner* element, either identical or almost identical to the copy present at w^{pch}. In common with w^{pch}, all four insertions are flanked by a presumed duplication of the dinucleotide TA. The precise location and orientation of each *mariner* insertion relative to the white gene are presented in Figure 4.

The w^{a49} allele contains a *mariner* insertion only 12 bp downstream from the w^{pch} insertion site, yet it shows a markedly different phenotype (*i.e.*, brown *vs.* peach). The orientation of *mariner* in this allele is the same as for the w^{pch} allele—that is, with the putative transcription unit of *mariner* in the opposite orientation to that of *white*. The allele with a bleached-white phenotype, w^{a45}, results from insertion of *mariner* into the second intron, 24 bp from the site of the *copia* insertion in the *white-apricot* allele in *D. melanogaster* (O'HARE *et al.* 1984). In this case *mariner* is also inserted with its putative transcription unit oriented in the opposite direction to that of the *white* gene. The w^{a41} allele contains *mariner* in a position that would place it in the fourth exon of the *white* gene by comparison to the intron-exon boundaries suggested for *D. melanogaster* (O'HARE *et al.* 1984). However, the w^{a41} allele does not exhibit a bleached-white phenotype, as might be expected of an insertion into an exon. It is phenotypically indistinguishable from the w^{a23} allele, in which the *mariner* insertion is just inside the third intron, and the insertion points of the two alleles are only 10 bp apart and in the same orientation. The w^{a41} and w^{a23} alleles both show temperature sensitive eye-color phenotypes: at 25° the eyes are orange in color, whereas at 18° the eye color is brown. On detailed inspection of the sequence flanking the w^{a41} insertion, it can be seen that the sequences of the two species differ with respect to the putative intron acceptor site, which is only 6 bp upstream of the insertion site. The A of the AG in *D. melanogaster* is substituted with T in *D. mauritiana*, suggesting that the intron-exon boundaries in the two species may differ (see Table 1). Thus the *mariner* insertion at w^{a41} may not disrupt an exon, which may explain why the phenotype is not bleached-white. Additional inspection of the region also reveals that, relative to *D. melanogaster*, *D. mauritiana* contains a deletion of 5 bp, as well as a single base pair insertion, both on the 3′ side of the *mariner* insertion site (data not shown). These differences would be rendered relatively insignificant if the intron acceptor site were moved downstream in *D. mauritiana*. There are two AG sequences, 24 and 38 bp downstream of the mutated acceptor site in *D. mauritiana*, the second of which would give the correct reading frame for the *white* gene (data not shown). Further work is required to resolve the possibility that these very closely related species may have different splicing patterns for the *white* gene product.

Germline revertant alleles derived from *mariner* insertions at *white*: Phenotypically wildtype alleles appear in *Mos1* strains at frequencies exceeding 2%. A number of phenotypically wild-type germline revertants were sequenced in order to determine whether the reversion to wildtype results from precise excision of *mariner*. Wild-type germline revertants of various *white* alleles, including w^{pch}, were collected from subcultures and made homozygous. DNA was prepared from these strains and polymerase chain reactions were carried out using primers flanking the *mariner* insertion sites. DNA sequences were determined for several of these revertants, and the data are shown in Table 2. In all cases both strands of the DNA sequence were obtained, and at least 50 bp either side of the insertion site were sequenced. The sequence data

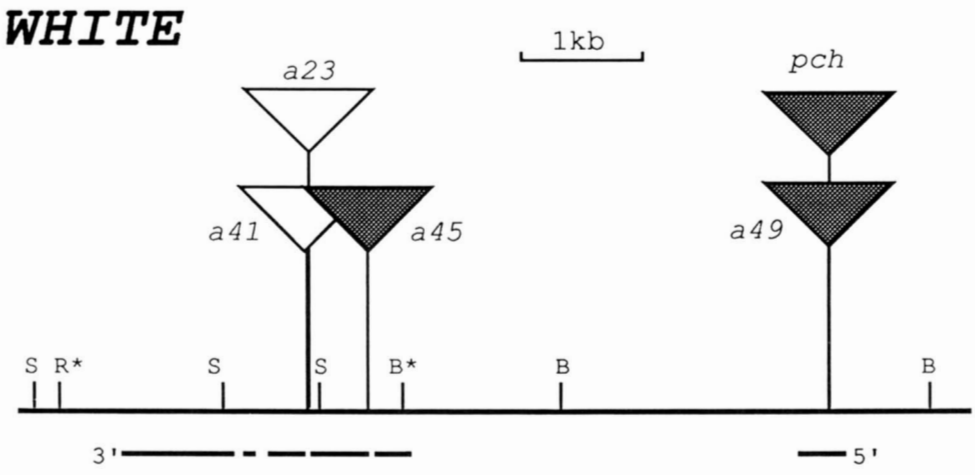

FIGURE 4.—Insertion sites of *mariner* within the *white* locus. The horizontal lines under map represent approximate positions of putative exon sequences, taken from O'HARE *et al.* (1984). Filled triangles represent *mariner* elements oriented against *white* transcription.

TABLE 2

Germline excision products from *mariner* induced *white* alleles

Allele	Phenotype	Sequence
Wild type	Wild type	–TTGATGGCG**TA**AACCGCTTG–
w^{pch}	Peach	–TTGATGGCG**TA*ccaggt....mariner....acctga*TA**AACCGCTTG–
Revertants (n = 10)	Wild type	–TTGATGGCG**TA*tga*TA**AACCGCTTG–
Revertants (n = 7)	Wild type	–TTGATGGCG**TA*cca*TA**AACCGCTTG–
Revertant	Wild type	–TTGATGGCG**TA*ga*TA**AACCGCTTG–
Revertant (w^{pchr19})	Brown	–TTGATGGCG**TA*cca*TAA*ccatga*TA**AACCGCTTG–
Wild type	Wild type	–CTTCGTGTTT**TA**ATTGATGGC–
w^{a49}	Brown	–CTTCGTGTTT**TA*ccaggt....mariner....acctga*TA**ATTGATGGC–
Revertants (n = 4)	Wild type	–CTTCGTGTTT**TA*tga*TA**ATTGATGGC–
Wild type	Wild type	–AAACTGGGTT**TA**CAAATAGTA–
w^{a45}	White	–AAACTGGGTT**TA*ccaggt....mariner....acctga*TA**CAAATAGTA–
Revertants (n = 2)	Wild type	–AAACTGGGTT**TA*cca*TA**CAAATAGTA–
Revertants (n = 2)	Wild type	–AAACTGGGTT**TA*tga*TA**CAAATAGTA–
Wild type	Wild type	–ATCTTGCAT**TA**CATCTCAAC–
w^{a41}	Orange	–ATCTTGCAT**TA*ccaggt....mariner....acctga*TA**CATCTCAAC–
Revertants (n = 2)	Wild type	–ATCTTGCAT**TA*tga*TA**CATCTCAAC–
Revertant	Wild type	–ATCTTGCAT**TA*cca*TA**CATCTCAAC–

Sequences are written such that the leftmost sequence is that flanking the 5′ terminus of the inserted *mariner* element. Sequences from *mariner* are in bold face lower case type. Presumptive duplications of *white* are written in bold face type.

from the revertants suggest that excision of *mariner* is rarely, if ever, precise. In all cases the presumptive 2 bp duplication of the dinucleotide TA was preserved. Furthermore in most relevant alleles, three base pairs, corresponding to either the 5′ or the 3′ end of *mariner*, were left behind at the insertion site, flanked by the 2 bp duplication. Since phenotypically wildtype revertants comprise the vast majority of visible *mariner* excision events (over 99%), it appears that these nearly precise excisions of *mariner* are the most common germline reversion events in the presence of *Mos1*.

Somatic reversion of w^{pch}: As is the case for the phenotypically wild-type germline revertants, somatic excision events also appear to generate a DNA fragment of wild-type size (see BRYAN and HARTL 1988). Sequences of somatic reversion events from w^{pch} were determined using the polymerase chain reaction. Because there are multiple reversion events in each fly, each sequence represents a consensus of an unknown number of events. By using maternal-effect mosaic flies, in which excision events are few in number, but early in development, it was possible to obtain DNA sequences for somatic excision events. The DNA sequence of the predominant event is identical to that found in the germline (*i.e.*, TATGATA). This does not preclude the occurrence of other events at lower frequency. These data are based on sequence analysis of 12 mosaic flies. It is worth noting that the somatic reversion events generate sectors that are clearly not wild type in eye color (see BRYAN, JACOBSON and HARTL 1987, Figure 1), whereas the identical germ line event gives an allele capable of producing wild-type eye pigmentation. The reasons for this difference in expression are not entirely clear.

Partial phenotypic revertants of w^{pch}: Strains carrying the w^{pch} allele give rise to various mutant derivatives in the presence of *Mos1*. Partial phenotypic

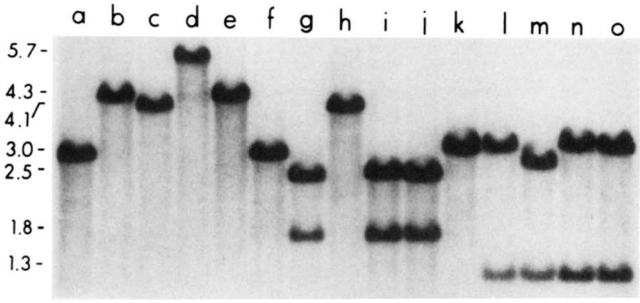

FIGURE 5.—Southern blot analysis of partial revertant derivatives of w^{pch}. DNAs were digested with *Bam*HI (lanes a–e), *Bam*HI and *Sal*I (lane f–j), *Bam*HI and *Sst*I (lanes k–o). DNAs were probed with the 3.0-kb *Bam*HI fragment (probe b). Lanes are as follows: lanes a, f, k, wild type; lanes b, g, l, w^{pch}; lanes c, h, m, w^{pchd5}; lanes d, i, n, w^{pchd7}; lanes e, j, o, w^{pchd4}.

revertants have eye colors intermediate between w^{pch} and wild type and occur at a frequency of approximately 0.1%. Southern blot analysis of several partial revertants showed that most fell into two classes: the *mariner* element was either missing or still present. This suggests that within each class the most common events leading to changes in phenotype were due to fairly small changes not resolved in conventional blots. In order to study derivative alleles at the sequence level, the polymerase chain reaction was used to amplify DNA sequences at the *mariner* insertion site. PCR reactions were performed using various combinations of primers from *mariner* and *white* to permit sequencing of regions at the termini of the transposon as well as flanking *white* DNA.

The Southern blot in Figure 5 shows a sample of three partial revertant alleles derived from w^{pch} that retain the *mariner* element. In this experiment, DNAs from five strains—wild-type, w^{pch}, and three mutant derivatives of w^{pch} (w^{pchd4}, w^{pchd5}, and w^{pchd7})—were digested with *Bam*HI, *Bam*HI-*Sal*I, and *Bam*HI-*Sst*I. The *mariner* transposon carries one *Sal*I site and one *Sst*I site, located 349 and 787 bp from the 5′ end of the element, respectively. DNA from the w^{pch} strain gives the characteristic pattern of two bands when probed with the 3.0-kb *Bam*HI fragment from *white* (probe b), consistent with the presence of a single site for *Sal*I and *Sst*I within the inserted DNA (Figure 5, lanes b, g and l). The wild-type allele contains no *Sal*I or *Sst*I sites within this fragment and therefore shows only a single hybridizing band (lanes a, f and k). The w^{pchd4} revertant is representative of revertant alleles that gave a pattern of bands identical to w^{pch} on Southern blots, suggesting that the phenotypic change is due to a relatively small change at the sequence level (lanes e, j and o). Sequencing revealed that w^{pchd4} contains a five base-pair deletion: a single nucleotide from the 5′ end of *mariner* is missing, as are the four adjacent base pairs of *white* sequence immediately flanking the w^{pch} insertion; these include one copy of the flanking TA duplicated on insertion.

Other partial revertants that retain the *mariner* element result from other types of event. Each of the partial revertant alleles shows reduced somatic instability in the presence of *Mos1*, and a phenotype less extreme than that of w^{pch}. Only two of the alleles, w^{pchd5} and w^{pchd7}, have given germline derivatives in the presence of *Mos1*, albeit at a much lower frequency than observed in w^{pch}. Southern blot analysis of w^{pchd5} (lanes c, h and m) revealed only one hybridizing band for *Sal*I-digested DNA, indicating that the *Sal*I site present in *mariner* has been lost in this allele. The presence of the *Sst*I site (lane m) indicated that part of *mariner* was still present. Sequencing showed that w^{pchd5} results from an internal deletion of 285 bp, beginning at position 136 within the *mariner* element. The allele w^{pchd7} has an eye-color considerably darker than w^{pch} (brown versus peach), and it has generated two derivatives that exhibit the original w^{pch} phenotype and pattern of hybridizing bands (data not shown). The genetic results suggest that the w^{pchd7} allele (lanes d, i and n) contains a second *mariner* element. In Southern blots with w^{pchd7}, the size of the *Bam*HI fragment is increased by approximately 1.3 kb with respect of w^{pch} (lane d), and yet the patterns of bands obtained in restriction digests with *Bam*HI/*Sal*I or *Bam*HI/*Sst*I are virtually identical to those seen in w^{pch} (lanes i and n). These results indicate that the second *mariner* element in the w^{pchd7} allele is inserted in the same orientation, either very close to, or just inside, the original w^{pch} *mariner* insertion. Sequencing of PCR-amplified fragments from various sites in the w^{pchd7} allele confirmed the insertion of a second *mariner* element into the *mariner* element already present at w^{pch}. The insertion site is 38 bp from the 5′ terminus of the w^{pch} *mariner*. A derivative of w^{pchd7}, resembling w^{pch} in phenotype, has lost the internal *mariner* element, but the w^{pch} element left behind contains the characteristic TATGATA "footprint" seen in wild-type revertants of w^{pch}. The structures of the alleles w^{pchd4}, w^{pchd5}, and w^{pchd7} are shown diagrammatically in Figure 6.

The w^{pchr19} allele is representative of revertant alleles with a pattern of bands identical to the wild-type allele but without wildtype eye color. In this case, *mariner* excision left 15 additional base pairs at the original insertion site, including sequences from both ends of the transposon as well as duplicated *white* sequence (see Table 2). The phenotype of this allele is a very dark red-brown, and a pseudopupil is visible, particularly in older flies.

Extreme mutant derivatives of w^{pch}: Southern blot analysis also showed that more extreme mutant derivatives (bleached-white or almost bleached-white eye color) usually resulted from imprecise excision of *mariner* from w^{pch} with the accompanying deletion of some flanking *white* sequences. In Figure 7 (lanes c, d and

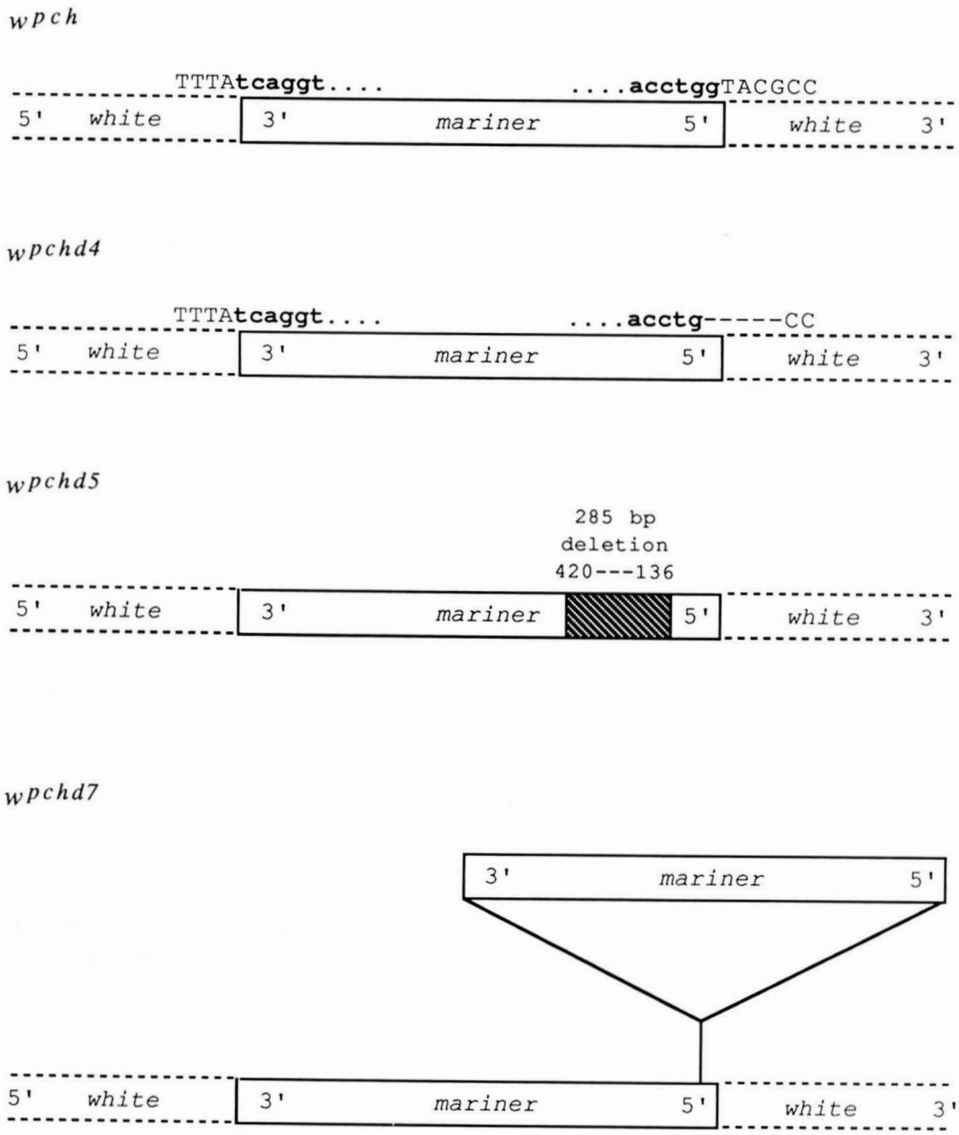

FIGURE 6.—Molecular structures of mutant derivatives of w^{pch}. In w^{pchd4}, 5 bp have been deleted, 1 bp from *mariner* and 4 bp from *white*.

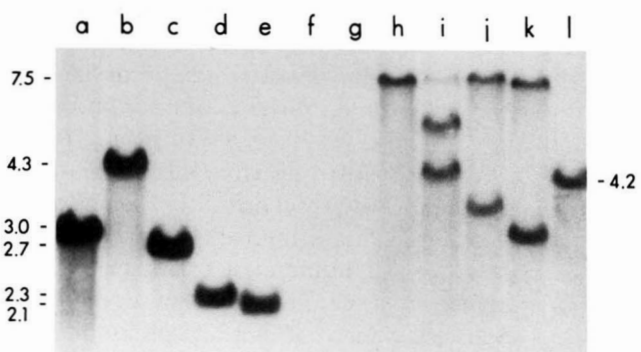

FIGURE 7.—Southern blot analysis of null derivatives of w^{pch}. DNAs were digested with *Bam*HI and probed with the 3.0-kb *Bam*HI fragment (probe b). Lanes are as follows: a, wild type; b, w^{pch}; c, w^{pchn1}; d, w^{pchn2}; e, w^{pchn3}; f, w^{pchn4}; g, w^{pchn5}; h, w^{pchn6}; i, w^{pchn7}; j, w^{pchn8}; k, w^{pchn9}; l, w^{pchn10}.

e) are some alleles showing deletions of part of the *Bam*HI fragment. These deletions vary in size from 300 to 900 bp. However, there are exceptional cases where it appears that flanking *white* DNA sequences have been deleted from both sides of the w^{pch} insertion site. The *Bam*HI fragment used as probe flanks the w^{pch} insertion site by 2300 and 800 bp, and this fragment has been deleted entirely in two derivatives (lanes f and g). This result has been confirmed on other Southern blots and is not simply due to failure of the probe to hybridize (data not shown). There are also derivatives that appear to have arisen from more complex events leading to the rearrangement of *white* sequences (lanes h–l), and at least one that retains a low level of somatic instability in the presence of *Mos1* (lane i), implying that *mariner* may still be inserted. This allele has given rise to one germline derivative, which does not have a null phenotype. In all cases except in the allele that retains its ability to revert, a *Bam*HI-*Sal*I digest gives the same pattern of bands as a *Bam*HI digest alone (data not shown), indicating that part and possibly all of *mariner* has been lost from the

insertion site. Some of these deletion alleles show extremely low levels of eye pigmentation in females, but not in males, suggesting a reduction or elimination of dosage compensation. Lethal alleles with bleached-white phenotypes, presumably arising through large deletions of flanking sequence, have been detected on two occasions. However, such alleles are difficult to maintain in *D. mauritiana*, owing to the complete lack of balancer chromosomes, and so it has not been possible to analyze them at the molecular level.

DISCUSSION

The *mariner* transposable element system differs from P/M hybrid dysgenesis in that its effects are manifest within mariner-bearing strains without the need for crossing strains possessing active *mariner* elements with strains lacking them. At present we have no evidence to suggest that the activity of *mariner* is increased in progeny of crosses between *Mos1*-containing strains and strains lacking *mariner*, suggesting that *mariner* may have no equivalent of the cytotype phenomenon observed with *P*. In *D. mauritiana*, this type of cross is difficult to perform since all strains in our possession carry *mariner* in high copy number. In crosses between strains of *D. simulans*, no increased activity of *mariner* has been detected when one of the strains is free of *mariner* elements (G. BRYAN, unpublished observations). However, in this case the strains with *mariner* have relatively low copy number, so any effects may be undetectable. Furthermore, while more work is required to address the activity of *Mos1* in the presence of varying numbers of *mariner* elements, the *Mos1* factor still manifests its effects when *mariner* is present in high copy number. The *mariner* transposable element system is likely to be a selective force within natural populations since it does not require a hybridization event for its activation. Because of their mutational effects, *Mos* factors might be disfavored in natural populations. On the other hand, such selection may be partly offset by the ability of *Mos* factors to replicate and transpose in the genome.

The frequency of mutations observed in this study was approximately 1 visible mutation per 4000 flies. This is probably an underestimate of the rate of mutation due to the transposition of *mariner*. There are many unknown parameters, such as the *mariner* transposition rate and the proportion of observed mutations that are actually due to the insertion of *mariner*. A number of the mutant alleles did arise as clusters of mutant males, suggesting the possibility of premeiotic events. However these apparent clusters could have resulted from a single heterozygous mutant female in the previous generation. Of the seven alleles investigated at the molecular level, all were found to contain *mariner* elements. However the cloned alleles were chosen on the basis of their mosaic phenotypes and so were not an unbiased sample. Some of the mutant strains that have not been investigated at the molecular level show germline instability, suggesting that *mariner* may be inserted into these alleles. It is also possible that some of the stable alleles are the result of secondary mutations due to the excision of *mariner*, with the accompanying deletion or rearrangement of flanking sequences. The apparent mutation rate is a minimum estimate since an unknown fraction of mutations are in genes that are not cell-autonomous and so would be suppressed by somatic excision of *mariner* in the presence of *Mos1*.

The range of phenotypes generated by the insertion of *mariner* at various positions in the *white* locus is of some interest. The allele w^{a41} is interesting in that *mariner* is possibly inserted into an exonic sequence, but does not give rise to a null phenotype. The phenotype of this allele is identical to that of allele w^{a23}, in which *mariner* is inserted 10 bp upstream into an intron. This raises the possibility that *D. mauritiana* uses an alternative splice site relative to *D. melanogaster*, whereby both insertions would be in the same intron. This would help to explain the identical phenotypes of the alleles and also rationalize sequence differences between the species in this region. The allele w^{a45} contains *mariner* inserted into an intron, yet exhibits a null phenotype. In this case the *mariner* element is transcribed in the opposite direction to *white*. The splicing of the intron in w^{a45} may be prevented by the presence of the *mariner* element, or the insertion of *mariner* may generate a transcription termination signal. Overall, the data from insertion sites of *mariner* suggest little insertion specificity for *mariner*. All insertions studied appear to be flanked by a duplication of the dinucleotide TA, but they have very little else in common. More flanking sequences will need to be analyzed before any consensus sequence is likely to emerge. The apparent lack of sequence specificity required for *mariner* insertion suggests that this transposon may turn out to be generally useful for studies of gene expression.

Our data suggest that *mariner*, like some other *Drosophila* transposons, does not excise precisely. In approximately 30 germline reversion events sequenced, no case of precise excision of *mariner* has yet been recovered. Most w^{pch} *mariner* excisions leave 3 bp of the transposon at the insertion site, along with the 2 bp of host sequence duplicated upon insertion. If the 5 bp of DNA remaining after *mariner* excision is the general case, then excision will not yield wild-type revertants when *mariner* is inserted into the coding region of a gene. However, at least six of seven *mariner* insertions studied at the molecular level are in noncoding regions. This propensity to insert into noncoding regions of genes is also seen with the *P*

element (ENGELS 1989). It appears that the predominant excision event of *mariner* in somatic cells is qualitatively the same as the most common germline event. This being the case, the phenotypic differences between germline and somatic excision products require some consideration. These differences may be due to a requirement for a structurally wildtype *white* gene before the period in development during which *white* transcription occurs. Somatic excision does sometimes yield sectors with wild-type eye pigmentation, and these are normally very early events yielding large sectors.

Mariner excision products in the germline and soma can be compared with products generated by other transposable elements. For example, the *Caenorhabditis elegans* transposon *Tc1* usually excises imprecisely in germline and soma. In somatic tissue, 11 of 20 excision products retained either a 3- or 4-bp insert, the TA duplicated upon insertion, as well as 1 or 2 bases of *Tc1* sequence (RUAN and EMMONS 1987, EIDE and ANDERSON 1987); the remaining nine excisions were precise. Of 13 wild-type germline revertants of *Tc1*-induced mutations in the *unc-54* and *unc-22* genes, only one was a precise excision. The most common events were the insertion of either 4 or 6 bp, including the duplicated target sequence and 2 bp from either or both ends of *Tc1* (KIFF *et al.* 1988; EIDE and ANDERSON 1988). (Interestingly the *unc-22* revertants showed larger insertions in two out of four cases, suggesting that the site of insertion may influence the excision process.) The observation that transposon sequences remain after excision suggest that *Tc1* and *mariner* may excise by mechanisms involving DNA cuts within the transposon. Excision of the *Drosophila P* element is also usually imprecise; most excision events leave *P*-element sequences—usually less than 40 bp in size—inserted at the donor site. Although the *P* element has been observed to excise precisely, these data are based on insertions into coding regions, and so the frequency of precise excision is probably biased upward by selection for the wild-type phenotype (O'HARE and RUBIN 1983). In maize, the *Ac/Ds* transposons are generally precisely excised, although all or most of the 8 bp duplication of the target site is left behind (SACHS *et al.* 1983; WECK *et al.* 1984; SUTTON *et al.* 1984; POHLMAN, FEDOROFF and MESSING 1984; BAKER *et al.* 1986; VAN SLUYS, TEMPE and FEDOROFF 1987). The *Spm* element generates a 3-bp duplication upon insertion, which upon excision is usually left, often with the substitution of one of the bases immediately flanking the insertion site (SAEDLER and NEVERS 1985; SCHWARZ-SOMMER *et al.* 1985).

The sequence data from mutant derivatives of w^{pch} is also quite informative. For example, the allele w^{pchd5} contains a *mariner* transposon with a 285 base-pair internal deletion. Judging from the low frequency of deleted elements found in natural populations, the generation of deletions internal to *mariner* is evidently a rare event (K. MARUYAMA, personal communication). This length uniformity of *mariner* contrasts sharply with *P*, where deleted elements are common, but is similar to the situation with *Tc1* in nematodes, in which deleted elements are rarely found. The deleted *mariner* in w^{pchd5} arose, in all probability, at the w^{pch} site and not as a result of some aberrant transposition event into the *white* locus. The greatly reduced rate of excision of this allele, relative to w^{pch}, suggests the possibility that excision may require sequences internal to the transposon, which would partly explain the length homogeneity. A second informative derivative of w^{pch} is the allele w^{pchd4}, the sequence of which suggests that deletion of a single base pair from the 5′ inverted repeat of *mariner* is sufficient to prevent or greatly reduce its excision in germline and soma. The deleted base is one of four in the inverted repeat that is mismatched with the opposite end of the *mariner* transposon. Also of interest in this allele is that the 4 bp of *white* that are deleted result in an eye color phenotype considerably darker than the w^{pch} allele. Two of the missing base pairs are the duplicated TA, which are also possibly important for excision. In the allele w^{pchd7} there is an insertion of another *mariner* element into the element already present in w^{pch}. This is a rare example of the insertion of one transposon into another of the same type, although cases of transposons inserting into unrelated elements have been reported (GEYER, GREEN and CORCES 1988). In such cases the secondary insertions were detected by their suppressive effect on the mutation caused by the initial insertion. The w^{pchd7} allele has a much darker eye color than the w^{pch} allele, indicating partial suppression in this case also. The w^{pchd7} allele reverts to a w^{pch}-like allele, which in turn reverts to a wild-type phenotype at low frequency. It does not appear that w^{pchd7} can revert to wild type in a single step. This lack of excision of both transposons simultaneously may be due to the increased overall size of the insertion, or alternatively, to the disruption of the outer *mariner* element by the second insertion. Although somatic mosaics are seen, one would expect the excision of the inner *mariner* element to generate peach-colored sectors that may not be detected on the darker background eye color. The mosaic sectors that are observed are usually darker, which may be due to somatic excision of both elements somewhat imprecisely, or, alternatively, the excision of mismatched ends of the transposon. These results indicate that the phenotype of the w^{pch} mutation results in part from features of *mariner* that are altered in the mutant derivative, rather than simply from the interruption of *white* sequences.

The bleached-white derivatives of w^{pch} show a varied range of molecular events, from small deletions to what appear to be fairly extensive rearrangements of the DNA sequences in and around the *white* gene itself. Of particular interest is that such events can generate alleles with lethal phenotypes, suggesting that the excision of *mariner* can cause deletions of considerable size at low frequency, with the further implication that *mariner*-induced events may cause extensive genomic rearrangement. (Some of these lethal chromosomes may result from the reinsertion of *mariner* at new sites in the *X* chromosome.) It is evident that *mariner* excision can bring about the deletion of DNA from both sides of the insertion, a fairly unusual observation. This type of event has been seen with Tam*3* in *Antirrhinum majus* (MARTIN, MACKAY and CARPENTER 1988). It would be interesting to know whether there are *mariner* sequences present at the breakpoints of the *mariner*-induced deletions.

Mutagenic effects of transposable elements have been observed in most organisms studied genetically. The use of strains in which transposons are known to be transpositionally active has enabled the cloning of genes by transposon tagging, as well as the study of the genes in which the transposons are inserted. In the *mariner* system we are using strains in which *mariner* elements are transposing in response to the *Mos1* factor, most likely an autonomous *mariner* element analogous to the 2907-bp *P* element. Our results suggest that *mariner* has the potential to be a useful tool for mutagenesis in *Drosophila* and the tagging of genes that are difficult to clone using *P* or *hobo* elements. Both the w^{pch} and *Mos1 mariner* elements have successfully been introduced into the genome of *Drosophila melanogaster*, which lacks *mariner* in all strains so far tested (D. GARZA, M. MEDHORA, A. ROGA and D. HARTL, manuscript in preparation). The introduction of *mariner* into *D. melanogaster* will permit useful studies on the population dynamics of the increase in copy number of this transposable element, as well as the use of *mariner* for transposon tagging. The high rate of somatic excision of *mariner* in *Mos1* strains may permit the rescue of insertions that have lethal or sublethal phenotypic effects. Also, by utilizing the sophisticated genetics of *D. melanogaster*, whereby mutation screens in which *Mos1* can be segregated away from the targeted chromosomes can be performed, it should be possible to screen for *mariner*-induced mutations in genes with non-cell-autonomous modes of expression.

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