Molecular structure of a somatically unstable transposable element in *Drosophila*

(white locus/molecular cloning/DNA sequence/*mariner*/somatic mutation)

JAMES W. JACOBSON*, MEETHA M. MEDHORA, AND DANIEL L. HARTL†

Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110-1095

Communicated by J. E. Varner, August 15, 1986

ABSTRACT A transposable element has been isolated from an unstable white mutation in *Drosophila mauritiana*, a sibling species of *Drosophila melanogaster*. The unstable white-peach (w^{pch}) allele exhibits a spectrum of germ-line and somatic mutability more similar to insertion mutations in maize and in the nematode *Caenorhabditis elegans* than has been reported for insertion mutations in *Drosophila*. The inserted element *mariner* is 1286 nucleotides long and has terminal inverted repeats. The element contains a single open reading frame encoding 346 amino acids. A duplication of 2 base pairs of white sequence is present at the insertion site. *Mariner* is present in approximately 20 copies in the *D. mauritiana* genome, is present from 0 to 7 copies in other members of the sibling species group, and is apparently absent from the genome of *D. melanogaster*.

Transposable elements comprise a significant fraction of the *Drosophila melanogaster* genome. Insertions of transposable elements have been shown to be the cause of many unstable mutations in this species. The pattern of genetic instability of these mutations, although peculiar to the type of element inserted, is largely limited to germ-line tissue, with somatic events being only rarely observed (1). In contrast to *Drosophila* transposons, germ-line and somatic instability are common features of transposable elements in maize (2) and in the nematode *Caenorhabditis elegans* (3–5).

Many of the transposable elements found in *D. melanogaster* are also present in closely related species, such as the *Drosophila* species: *D. simulans*, *D. mauritiana*, *D. sechellia*, *D. erecta*, *D. orena*, *D. yakuba*, and *D. teissieri* (6). The number of copies of a particular transposable element present in the genome may differ dramatically in related species. For example, *D. simulans* and *D. mauritiana* have been shown to contain substantially less middle repetitive DNA than does *D. melanogaster* (7), and *D. erecta* apparently contains families of repetitive elements not present in *D. melanogaster* (8). However, little is known about the characteristics of elements present in species related to *D. melanogaster* that are not present in *D. melanogaster* itself. In this paper we report the isolation and characterization of a transposable element that is present in many copies in the genome of *D. mauritiana* but is apparently absent in *D. melanogaster*.

We have designated the *D. mauritiana* transposable element *mariner*. It was discovered as a repetitive DNA sequence inserted in the gene for white eyes (*w*). The mutant eye color is designated peach, and the insertion mutation itself has been designated white-peach (w^{pch}) (9, 10). The unstable w^{pch} allele exhibits genetic characteristics typically associated with insertions of transposable elements in *D. melanogaster*. For example, w^{pch} reverts to wild type at a frequency of about 10^{-3} per generation. It also produces null

derivatives, characteristic of deletions, at about the same frequency (9). In addition to these features, w^{pch} exhibits a distinctive pattern of mutability for *Drosophila* insertion mutations in that it remains unstable in both germinal and somatic tissue (9, 10).

The white-eyes gene of *D. melanogaster* has been the subject of extensive genetic and molecular analysis (11), the wild-type allele has been cloned, and its nucleotide sequence has been determined (12–15). Several insertion mutations in the gene have been characterized at the molecular level, and their effects on gene expression have been determined (11, 15–18).

We have used cloned white DNA sequences from *D. melanogaster* to identify and isolate homologous sequences from the mutant w^{pch} allele in *D. mauritiana*. From these white sequences the *mariner* transposable element was isolated, and its nucleotide sequence and structural characteristics have been determined.

MATERIALS AND METHODS

Fly Strains and Culture Methods. The origin and genetic characterization of w^{pch} have been described (9, 10). *D. mauritiana* isofemale lines G102, G122, G190, G193, G194, G206, G284, and G295 were provided by R. C. Woodruff and R. F. Lyman. *D. sechellia*, *D. erecta*, *D. yakuba*, and *D. teissieri* were provided by the Bowling Green Stock Center. *D. mauritiana* w^+ , *D. simulans*, and *D. melanogaster* Oregon-R are wild-type strains maintained in our laboratory. Flies were cultured at 25°C in standard cornmeal/agar *Drosophila* medium or Formula 4-24 instant medium (Carolina Biological, Burlington, NC).

Bacterial Strains, Cloning Vectors, and Media. *Escherichia coli* strain Q358 or Q364 was used as host for bacteriophage λ 1059 (19). The plasmid pBR322 (20) and recombinant plasmid derivatives were maintained in *E. coli* strain HB101. Plasmids containing *D. melanogaster* white sequences were provided by P. M. Bingham (coordinates -0.5 to $+3.3$ and $+3.3$ to $+6.8$ on white restriction map, see ref. 13) and G. M. Rubin (plasmid pm12.8). The phage vectors M13mp18 and M13mp19 (21) and recombinant derivatives were grown in *E. coli* strain JM107 (21). Methods for growth of phage and plasmids have been described (22).

General Biochemical Methods. Methods for isolation of DNA (22–24), restriction digests, gel electrophoresis (22), Southern transfers (25), recovery of DNA (22, 26), filter hybridization (27), and nick-translation of probes (28) have all been described. Genomic libraries were constructed using the vector λ 1059 (19). Genomic DNA was partially digested with *Sau*3A and fragments of 15–20 kilobases (kb) were ligated into the *Bam*HI site of the cloning vector λ 1059 (19).

Abbreviations: bp, base pair(s); kb, kilobase pair(s).

*Present address: Department of Genetics, North Carolina State University, Box 7614, Raleigh, NC 27695.

†To whom reprint requests should be addressed.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

The recombinant phage were recovered by *in vitro* packaging with Gigapack (Vector Cloning Systems, San Diego, CA) and screened by the Benton and Davis method (29).

Sequencing of Nucleic Acids. Nucleotide sequences were determined by the chain-termination method of Sanger *et al.* (30) as modified by Barnes *et al.* (31). The sequencing reactions were initiated with either the universal M13 primer or 18-base synthetic oligonucleotide primers prepared with an Applied Biosystems (Foster City, CA) DNA synthesizer. The sequence of the w^+ region spanning the site of *mariner* insertion in w^{pch} was determined from plasmid pJJ3. Procedures for using plasmid DNA as the template for sequencing reactions have been described (32). Sequences were assembled and analyzed using an IBM personal computer and the Microgenie Sequence Analysis Program (33) as supplied by Beckman.

RESULTS

The w^{pch} Allele Contains an Inserted DNA Sequence. Preliminary Southern hybridizations (25) of DNA from w^{pch} and w^+ strains, digested with various restriction enzymes and probed with *D. melanogaster* white DNA, yielded the restriction maps in Fig. 1. Comparison of the alleles reveals that they differ only in the size of a *Xho* I–*Bam*HI fragment (coordinates +1.6 to +4 in the w^+ map). The difference in size is consistent with the presence of a 1.3-kb insertion of DNA in w^{pch} . Furthermore, by extending the analysis to a wild-type revertant of w^{pch} , we observed that the *Xho* I–*Bam*HI fragment was restored to the approximate size of 2.4 kb, which is characteristic of the w^+ allele (data not shown). From these results, we conclude that the w^{pch} allele contains 1.3 kb of inserted DNA that is lost from the gene upon reversion to wild type.

Molecular Cloning of the w^{pch} Insertion. Sequences containing white DNA were isolated from λ 1059 genomic libraries prepared from w^{pch} or w^+ DNA. Two of the phage— λ JJ4.1 from the w^+ library and λ JJw1.1 from the w^{pch} library—were mapped, and from them the appropriate *Bam*HI fragments were isolated and subcloned into pBR322 for detailed analysis. The cloned *Bam*HI fragments, present in plasmids pJJ1 and pJJ3, are indicated beneath the restriction maps in Fig. 1.

The Insertion in w^{pch} Is a Transposable Element. The 1.3-kb insertion in the w^{pch} gene exhibits several features that are characteristic of transposable elements in *D. melanogaster*.

First, the insert in w^{pch} occurred spontaneously, and wild-type revertants have lost the insert. Secondly, the element is present in multiple copies in the genome of *D. mauritiana*. Thirdly, the element demonstrates variation in number of copies and positions in the genome when compared among strains of a single species and among different species. These features strongly suggest that the DNA sequence inserted in the w^{pch} gene is a transposable element.

Variation in copy number of the transposable element among related species was demonstrated as follows. Genomic DNA from *D. mauritiana* (w^{pch} and w^+ strains), *D. sechellia*, *D. simulans*, *D. melanogaster*, *D. erecta*, *D. yakuba*, and *D. teissieri* was digested with *Bam*HI and *Hind*III, separated by agarose gel electrophoresis, and transferred to a nylon membrane. The membrane was hybridized sequentially, first with the 3.0-kb *Bam*HI fragment from pJJ3 (which contains only white sequences), and then with the 4.3-kb *Bam*HI fragment from pJJ1 (which contains the same white sequences plus the 1.3-kb insertion). The results of the hybridizations are shown in Fig. 2. Fig. 2A shows the pattern of hybridization with white DNA; the hybridization signals in the lanes containing w^{pch} and w^+ DNA are of the sizes predicted from Fig. 1, namely 4.3 kb and 3.0 kb, respectively. The sizes of fragments hybridizing with *D. melanogaster* genomic DNA are those predicted from the white restriction map in this strain (16).

The results shown in Fig. 2B clearly indicate that the 4.3-kb *Bam*HI fragment from w^{pch} contains a non-white inserted sequence that is highly repetitive in *D. mauritiana*, less repetitive in *D. sechellia*, *D. simulans*, *D. yakuba*, and *D. teissieri*, and apparently absent at this stringency of hybridization in *D. erecta* and *D. melanogaster*. We designate the inserted element as *mariner*.

It is possible that the experimental procedure summarized in Fig. 2 masks *mariner* copies present in *D. melanogaster* and *D. erecta*, if they are contained within restriction fragments that comigrate with fragments hybridizing to white sequences in the probe. To address this possibility, we carried out similar experiments using several strains of *D. melanogaster* (including strains recently isolated from wild populations) and the *D. erecta* strain. In these experiments, the hybridization probe was an internal restriction fragment of *mariner* (a 0.64-kb *Sal* I–*Sph* I fragment, described below). No detectable *mariner* hybridization signal, at this stringency, was observed in any of the *D. melanogaster* strains or in *D. erecta*, whereas a control lane containing *D. mauritiana*

FIG. 1. Restriction map of the *D. mauritiana* white locus region in w^+ and w^{pch} . The coordinate system is that of ref. 12. The telomere is to the left, and the centromere is to the right. Nick-translated probes used for the analysis covered regions -2.7 to -1.2 , -0.5 to $+3.3$, and $+3.3$ to $+6.8$. The probe spanning from -0.5 to $+3.3$ was used to screen the λ 1059- w^{pch} and λ 1059- w^+ genomic libraries. Positive λ clones isolated from the libraries had the same restriction map of the -0.5 to $+3.3$ region as shown here. The region cloned into plasmid pJJ3 is indicated beneath the w^+ restriction map, and the region cloned into plasmid pJJ1 is shown beneath the restriction map of w^{pch} .

FIG. 2. Autoradiograph of Southern transfer hybridization of genomic DNA from members of the *melanogaster* species subgroup. Each lane contains genomic DNA, digested with *Bam*HI and *Hind*III, from the following samples: lane 1, *D. mauritiana* w^{pch} ; lane 2, *D. mauritiana* w^+ ; lane 3, *D. sechellia*; lane 4, *D. simulans*; lane 5, *D. melanogaster*; lane 6, *D. erecta*; lane 7, *D. yakuba*; and lane 8, *D. teissieri*. The filter was hybridized with the 3.0-kb *Bam*HI fragment from plasmid pJJ3 containing only white sequences (A), and then rehybridized with the 4.3-kb *Bam*HI fragment from plasmid pJJ1, which contains the same white sequences plus the 1.3-kb insert (B). Size markers along the left margin represent λ -*Hind*III fragments in kb as follows from the top: 24, 9.5, 6.8, 4.3, 2.3, and 2.0.

genomic DNA gave a hybridization signal similar to the result in Fig. 2 (data not shown).

Variation in number of copies and genomic positions of the *mariner* element in *D. mauritiana* are indicated in Fig. 3. Genomic DNA from eight *D. mauritiana* isofemale lines was digested with *Bam*HI and *Hind*III, separated in agarose gels, and blotted to nitrocellulose. The blot was then hybridized with the 4.3-kb *Bam*HI fragment from plasmid pJJ1. Variation in genomic position of the inserted *mariner* sequence among different strains of *D. mauritiana* is apparent in Fig. 3. The *mariner* element, therefore, appears to be a mobile element, but one not normally present in the genome of *D. melanogaster*.

FIG. 3. Autoradiograph of Southern transfer hybridization of genomic DNA from *D. mauritiana* isofemale lines. Each lane contains genomic DNA, digested with *Bam*HI and *Hind*III and hybridized with the 4.3-kb *Bam*HI fragment from plasmid pJJ1. Strains are shown above the lanes. The arrow at right indicates the 3.0-kb w^+ *Bam*HI fragment that hybridizes to white sequences in the probe. Size markers along left margin represent λ -*Hind*III fragments in kb as follows from the top: 24, 9.5, 6.8, 4.3, 2.3, and 2.0.

Nucleotide Sequence Characterization of the *Mariner* Element. The inserted *mariner* element contained within the 4.3-kb *Bam*HI fragment in plasmid pJJ1 was identified through comparison of the restriction map of pJJ1 with that of pJJ3 (Fig. 1). In addition, restriction digests of pJJ1 were transferred to nitrocellulose filters and hybridized with nick-translated *D. mauritiana* genomic DNA to identify fragments containing repetitive sequences and those containing single copy sequences. These studies indicated that the *mariner* insert contains one *Sal*I site, which was used to separate the 4.3-kb *Bam*HI fragment into two *Bam*HI-*Sal*I fragments, 1.8 kb and 2.5 kb long, each terminating with the *Sal*I site from inside the transposon. A 0.75-kb *Pvu*II-*Sph*I fragment spanning the *Sal*I site in the transposon was also isolated. These fragments were subcloned into both M13mp18 and M13mp19 vectors (21) for the determination of the nucleotide sequence (Fig. 4).

The nucleotide sequence of both strands of the transposon was determined, as well as sequences from the white gene flanking the *mariner* element at each end (Fig. 5). In addition, 144 base pairs (bp) of sequence spanning the site of the *mariner* insertion was determined from plasmid pJJ3, containing w^+ DNA, by the method of double-stranded sequencing (32).

The *mariner* element inserted in the w^{pch} allele is 1286 nucleotides long and contains a terminal inverted repeat sequence of 28 bp. Comparison of the two inverted repeats reveals four mismatches out of the total 28 bp. The element features one open reading frame extending from nucleotide 172 to 1209. Translation of this open reading frame would yield a 346-amino acid translation product having a molecular weight of 40,855. At the insertion site in w^{pch} , there appears to be a duplication of 2 bp.

Although we have no direct evidence that this copy of *mariner* is transcribed and translated, the sequence shares several features with active genes. For example, there are sequences approximating 5'-promoter consensus sequences common to many eukaryotic genes (34), in particular the sequences CAACC at -74, CCATA at -54, and a "TATA" box (CATAA) at -29, relative to the potential initiation codon (see Fig. 5). Many reported eukaryotic genes contain a purine, usually adenosine, at the -3 position (35), relative to the start of translation, and *mariner* shares this feature. In fact, the putative *mariner* translational start context matches the consensus *D. melanogaster* sequence in the first 9 of 10 upstream nucleotides (D. Cavener, personal communication). Following the open reading frame, and including the stop codon, is a typical polyadenylation sequence (AATAAA). Finally, codon usage within the long open reading frame of *mariner* is typical of *Drosophila* genes as assessed by comparison (36) with the suggested reading frame of the transposable element *copia* (37) or with the *D. melanogaster* genes for alcohol dehydrogenase (38) or opsin (39).

The *mariner* sequence and the putative translation product were compared to those of reported transposons in *Drosophila* and other organisms by searching the Microgenie data bank. No homologies of any statistical significance were found for either the nucleotide or amino acid sequence.

Conservation of the *Mariner* Restriction Map. Most of the approximately 20 copies of the *mariner* element present in the *D. mauritiana* genome have the same restriction map as the element in w^{pch} . This conclusion is supported by two types of observations. First, the 0.64-kb *Sal*I-*Sph*I fragment internal to the transposon was isolated for use as a hybridization probe (Fig. 4). Various combinations of the restriction enzymes shown in Fig. 4 were used to digest genomic DNA, which was probed with the internal *mariner* fragment in Southern blots. The hybridization signal was located in

FIG. 4. *Mariner* restriction map and sequencing strategy (arrows). Use of the universal primer in sequencing reactions is indicated by the short vertical line at the base of the arrows. Other sequences were obtained using synthetic oligonucleotide primers.

fragments of the sizes predicted from the map shown in Fig. 4 (unpublished data).

The second approach to determine the size and structure of other *mariner* elements in the genome was to isolate recombinant phage clones from the w^{pch} library using the *Sal*I–*Sph*I fragment as a probe. Phage were selected in which the cloning site was not within the transposon; these phage contain the transposon flanked by unique genomic DNA. Restriction digests and blotting experiments with the flanking DNA were used to identify recombinant phage containing DNA from different locations in the genome. Four phage were chosen at random for further analysis. All four were found to have restriction maps identical to that of the *mariner* element inserted in the w^{pch} allele (unpublished data).

Mapping the Site of Insertion and the Nature of the Revertants. The DNA sequence of the white gene in *D. mauritiana* is closely conserved with that in *D. melanogaster* (unpublished data). From the sequence of the white DNA flanking the insert at w^{pch} , we were able to locate the precise point of insertion within the white transcription unit. The transposon at w^{pch} is inserted into the 5'-nontranslated leader region at nucleotide position +3757 and is near the site of insertion of an F-like element associated with the mutant w^h (white-honey) in *D. melanogaster* (15). The orientation of the inserted *mariner* element is opposite that of the direction of *w* gene transcription.

Since genetically stable wild-type revertants are recovered from w^{pch} (9, 10), it is likely that the *mariner* transposon is capable of excising with sufficient precision to restore wild-

type white activity. This hypothesis was tested by Southern blotting experiments using the 3.0-kb *Bam*HI fragment from plasmid pJJ3 as a probe. Among 18 independent wild-type revertants from w^{pch} , all showed restriction fragments that were identical in size to wild type within the limit of resolution of the technique (50–100 bp).

The w^{pch} mutation also produces mutant derivatives having white eyes (9). Some of these white-eye mutations contain substantial deletions within the 3.0-kb *Bam*HI fragment formerly containing the *mariner* insert. For example, one contained a deletion of ≈ 2.1 kb of white DNA and another a deletion of ≈ 200 bp. Other white-eye derivatives are indistinguishable from w^+ at the level of restriction fragment analysis and may contain undetected, small deletions of white sequences (data not shown). These results demonstrate that *mariner* can excise to restore gene function and can also generate deletions leading to null derivatives.

DISCUSSION

Mariner is a transposable element in *D. mauritiana* that is associated with an unstable white mutation. The genetic characteristics of the *mariner*-induced mutation (w^{pch}) and the lack of sequence homology to other *Drosophila* transposons suggest that *mariner* represents a newly described family of transposable elements in *Drosophila*. The observed distribution of *mariner* in the *melanogaster* species subgroup is consistent with phylogenies based on polytene chromosome banding pattern (6), studies of mitochondrial DNA (40),

```

1-CCAGGTGTACAAGTAGGGAATGTCGGTTTCGAACATATAGATGTCGCAACGTAATATTTACCGATTGTCATAAACTTTGACCTTGTGAAGTGCAACCTTGACTGTCGAA
115-CCACCATAGTTTGGCGCAAATTGAGCGTCATAATTGTTTCTCTCAGTGCAGTCAACATGTCGAGTTTCGTGCGGAATAAGAGCAAACGCGGACAGTATTAATTTCTGTTT
MetSerSerPheValProAsnLysGluGlnThrArgThrValLeuIlePheCysPhe

229-CATTGAAGAAAAAGCTGCGGAATCGCACCGAATGCTTGTGAAGCCTTTGGCGAACAAGTACCAACTGTGAAAAAGTGAACGGTGGTTTCAACGCTTCAAAAGTGGTGAT
HisLeuLysLysThrAlaAlaGluSerHisArgMetLeuValGluAlaPheGlyGluGlnValProThrValLysLysCysGluArgTrpPheGlnArgPheLysSerGlyAsp

343-TTTGACGTCGACGACAAAGAGCAGGAAACCGCCAAAAGGTACGAAGACGCGCAACTGCAAGCATTATTGGATGAAGACGATGCTCAACGCAAAAACAACTCGCAGAGCAG
PheAspValAspAspLysGluHisGlyLysProProLysArgTyrGluAspAlaGluLeuGlnAlaLeuLeuAspGluAspAlaGlnThrGlnLysGlnLeuAlaGluGln

457-TTGAAGTAAGTCAACAGCAGTTTCAATCGCTTGCAGAGATGGGAAAGATTGAGAAGTGGGTAGATGGGTGCCACATGAGTTGAACAGAGAGGAGATGGAGAGGCGCAAA
LeuGluValSerGlnGlnAlaValSerAsnArgLeuArgGluMetGlyLysIleGlnLysValGlyArgTrpValProHisGluLeuAsnGluArgGlnMetGluArgArgLys

571-AACACATGCGAAATTTTCTTTCAGATACAAAAGGAAGTCGTTTTGTCATCGTATCGTTACTGGCGATGAAAAATGGATCTTTTTTGTAGTCTAAACGTAAAAAGTCATAC
AsnThrCysGluIleLeuLeuSerArgTyrLysArgLysSerPheLeuHisArgIleValThrGlyAspGluLysTrpIlePhePheValSerProLysArgLysLysSerTyr

685-GTTGATCCTGGACAACCGCCACATCGACTGCTCGACCGAATCGCTTGGCAAGAAGACGATGCTCTGTGTTGGTGGGATCAGAGCGGTGTCATTACTATGAGCTCTTGAAA
ValAspProGlyGlnProAlaThrSerThrAlaArgProAsnArgPheGlyLysLysThrMetLeuCysValTrpTrpAspGlnSerGlyValIleTyrTyrGluLeuLeuLys

799-CGCGCGCAACCGTGAATACGGCACGCTACCAACAACAAATTGATCAATTTGAACCGTGCCTTCAGAGAAAACGACCGGAATATCAAAAAAGACAACAGGGTCATTTTCTC
ArgGlyGluThrValAsnThrAlaArgTyrGlnGlnGlnLeuIleAsnLeuAsnArgAlaLeuGlnArgLysArgProGluTyrGlnLysArgGlnHisArgValIlePheLeu

913-CATGACAACGCTCCATCACATACGGCAAGAGCGGTTTCGCGACAGCTTGGAAACACTCAATTGGGAAGTGTTCGCGATGCGGCTTACTCACCAGACCTGGCCCCATCCGATTAC
HisAspAsnAlaProSerHisThrAlaArgAlaValArgAspThrLeuGluThrLeuAsnTrpGluValLeuProHisAlaAlaTyrSerProAspLeuAlaProSerAspTyr

1027-CACATTTCGCTTCGATGGGACACGCACTCGCTGAGCAGCGCTTCGATCTTACGAAAGTGTGAAAAATGGCTCGATGAATGGTTTCGCGCAAAAGACGATGAGTTCTACTGG
HisLeuPheAlaSerMetGlyHisAlaLeuAlaGluGlnArgPheAspSerTyrGluSerValLysLysTrpLeuAspGluTrpPheAlaAlaLysAspAspGluLeuTyrTrp

1141-CGTGGAATCCACAAATTCGCCGAGAGATGGGAAAAATGTGTAGCTAGCGACGGCAAATACTTAGAATAAATGATTTTTTCTTTTCCACAAAATTTAACGTGTTTTTGATTAAA
ArgGlyIleHisLysLeuProGluArgTrpGluLysCysValAlaSerAspGlyLysTyrLeuGluEnd

1255-AAAAAACGACATTTTCATCTTGTACACCTGA-1286

```

FIG. 5. DNA sequence of the *mariner* element inserted at w^{pch} . This strand runs from distal to proximal with respect to the centromere. Numbering starts with position +1 at the 5' end of the putative *mariner* coding strand. Inverted repeats at the termini are underlined, as are potential promoter sequences. The amino acid sequence is shown beneath the proposed open reading frame. The nucleotide sequence context of the insertion in w^{pch} is as follows: 5' AATTGATGGCGTA/TAAACCGCTTGGGA 3', where the slash marks the insertion; the TA flanking the insert is a putative 2-bp duplication.

reproductive isolation and behavior (41–43), and the DNA sequence of the alcohol dehydrogenase gene (44). The apparent absence of *mariner* from the genome of *D. melanogaster* is particularly noteworthy since *D. mauritiana* has been shown to contain less total middle repetitive DNA and fewer copies of several identified transposable elements than *D. melanogaster* (7, 8). The simplest explanation for the present distribution of *mariner* within the species subgroup is that the element, present in a common ancestor, has been lost from lines leading to *D. melanogaster* and *D. erecta*.

The germ-line and somatic instability of w^{pch} more closely resemble the genetic characteristics of plant insertion mutations (2) than they do reported *Drosophila* insertion mutants (1). Furthermore, the overall structure and sequence organization of *mariner* are similar to that of other eukaryotic transposable elements that are unstable in somatic cells. The best known examples are the *Ac* element in maize (2, 45) and *Tcl* in *C. elegans* (46), which also contain inverted repeats and long open reading frames. It may be of interest that nucleotides 14–22 and 1267–1277 within the inverted repeats of *mariner* bear a remarkable similarity with the first 8 bp of the inverted repeats of the maize *Ac* element, and the mismatched terminal nucleotide in *mariner* also occurs in *Ac* (45). The *Drosophila* P element contains terminal inverted repeats (47), but is unstable only in germ cells (1, 48–49).

The presence of a long open reading frame suggests that *mariner* encodes a protein that functions in the transposition of the element. Although we have no direct evidence of transcription, the presence and position of appropriate transcriptional control signals within the element suggest that it could be transcribed and translated. In the absence of sequence data from other copies of the *mariner* element, it is difficult to determine whether the mismatches in the inverted repeats and the 2-bp target site duplication found here are characteristics of *mariner* in general or are peculiar to this particular copy. Conservation of restriction sites among other genomic copies of the element seems to indicate that the *mariner* element inserted at w^{pch} is representative.

The isolation and characterization of the *mariner* element present an exciting set of opportunities. Since fertile female hybrids can be obtained by crossing either *D. simulans* or *D. sechellia* with *D. mauritiana* (43), genes from the latter can be transferred across the species boundaries merely by repeated backcrossing. In this manner, the effect of various numbers of copies of the *mariner* element can be investigated. We have introduced the *mariner* element into the genome of *D. melanogaster* by P element-mediated transformation (unpublished data). The powerful genetic and molecular techniques available in *D. melanogaster* can thereby be used to determine whether the somatic instability of *mariner* is limited to *D. mauritiana* by some unidentified host–transposon interaction or whether it is a general feature of the *mariner* family. Additionally, the population dynamics of invasion of a transposable element into a genome, analogous to the transgenic infection of *D. simulans* with the P element from *D. melanogaster* (50), can be studied in detail.

We are grateful to R. C. Woodruff and R. F. Lyman for providing fly strains; to P. M. Bingham, V. Pirrotta, and G. M. Rubin for plasmids containing *D. melanogaster* white sequences; and to J. Kiff, K. Kondo, and L. Green for advice on sequencing. Thanks to D. Garza, R. DeSalle, D. Moerman, and N. J. Scavarda for helpful discussion and comments on an earlier draft of this manuscript. This work was supported by Research Grant GM33741 and Training Grant GM08036 (J.W.J.) from the National Institute of General Medical Sciences.

1. Rubin, G. M. (1983) in *Mobile Genetic Elements*, ed. Shapiro, J. A. (Academic, New York), pp. 329–361.
2. Federoff, N. V. (1983) in *Mobile Genetic Elements*, ed. Shapiro, J. A. (Academic, New York), pp. 1–63.
3. Emmons, S. W. & Yesner, L. (1984) *Cell* **36**, 599–605.
4. Eide, D. & Anderson, P. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1756–1760.
5. Moerman, D. G., Benian, G. M. & Waterston, R. H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2579–2583.
6. Lemeunier, F. & Ashburner, M. (1984) *Chromosoma* **89**, 343–351.
7. Dowsett, A. P. & Young, M. W. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4570–4574.
8. Dowsett, A. P. (1983) *Chromosoma* **88**, 104–108.
9. Jacobson, J. W. & Hartl, D. L. (1985) *Genetics* **111**, 57–65.
10. Haymer, D. S. & Marsh, J. L. (1986) *Dev. Genet.* **6**, 281–291.
11. Judd, B. H. (1976) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Novitski, E. (Academic, London), Vol. 1b, pp. 767–799.
12. Bingham, P. M., Levis, R. & Rubin, G. M. (1981) *Cell* **25**, 693–704.
13. Levis, R., Bingham, P. M. & Rubin, G. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 564–568.
14. Goldberg, M. L., Paro, R. & Gehring, W. J. (1982) *EMBO J.* **1**, 93–98.
15. O'Hare, K., Murphy, C., Levis, R. & Rubin, G. M. (1984) *J. Mol. Biol.* **180**, 437–455.
16. Zachar, Z. & Bingham, P. M. (1982) *Cell* **30**, 529–541.
17. Levis, R., O'Hare, K. & Rubin, G. M. (1984) *Cell* **38**, 471–481.
18. Pirrotta, V. & Brockl, Ch. (1984) *EMBO J.* **3**, 563–568.
19. Karn, J., Brenner, S., Barnett, L. & Cesarini, G. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5172–5176.
20. Bolivar, F., Rodriguez, R. L., Greene, P. J., Betlach, M. C., Heynecker, H. L. & Boyer, H. W. (1977) *Gene* **2**, 95–113.
21. Yanisch-Perron, C., Vieira, J. & Messing, J. (1983) *Gene* **33**, 103–119.
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
23. Lis, J. T., Simon, J. A. & Sutton, C. A. (1983) *Cell* **35**, 403–410.
24. Kuner, J. M., Nakanishi, M., Ali, Z., Drees, B., Gustavson, E., Theis, J., Kauvar, L., Kornberg, T. & O'Farrell, P. H. (1985) *Cell* **42**, 309–316.
25. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
26. Vogelstein, B. & Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 615–619.
27. Goodman, H. M., Olson, M. V. & Hall, B. D. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5453–5457.
28. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
29. Benton, W. D. & Davis, R. W. (1977) *Science* **196**, 180–182.
30. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
31. Barnes, W. M., Bevan, M. & Son, P. H. (1983) *Methods Enzymol.* **101**, 98–122.
32. Chen, E. Y. & Seeburg, P. H. (1985) *DNA* **4**, 165–170.
33. Queen, C. & Korn, L. J. (1984) *Nucleic Acids Res.* **12**, 581–599.
34. Myers, R. M., Tilly, K. & Maniatis, T. (1986) *Science* **232**, 613–618.
35. Kozak, M. (1984) *Nucleic Acids Res.* **12**, 857–872.
36. Staden, R. & McLachlan, A. D. (1982) *Nucleic Acids Res.* **10**, 141–156.
37. Mount, S. M. & Rubin, G. M. (1985) *Mol. Cell. Biol.* **5**, 1630–1638.
38. Benyajati, C., Spoerel, N., Haymerle, H. & Ashburner, M. (1983) *Cell* **33**, 125–133.
39. O'Tousa, J. E., Baehr, W., Martin, R. L., Hirsh, J., Pak, W. L. & Applebury, M. L. (1985) *Cell* **40**, 839–850.
40. Solignac, M. & Monnerot, M. (1986) *Evolution* **40**, 531–539.
41. Robertson, H. M. (1983) *Evolution* **37**, 1283–1293.
42. Coyne, J. A. (1985) *Nature (London)* **314**, 736–737.
43. Lachaise, D., David, J. R., Lemeunier, F., Tsacas, L. & Ashburner, M. (1986) *Evolution* **40**, 262–271.
44. Bodmer, M. & Ashburner, A. (1984) *Nature (London)* **309**, 425–430.
45. Doring, H. P. & Starlinger, P. (1984) *Cell* **39**, 253–259.
46. Rosenzweig, B., Liao, L. W. & Hirsch, D. (1983) *Nucleic Acids Res.* **11**, 4201–4209.
47. O'Hare, K. & Rubin, G. M. (1983) *Cell* **34**, 25–35.
48. Engels, W. R. (1983) *Annu. Rev. Genet.* **17**, 315–344.
49. Laski, F. A., Rio, D. C. & Rubin, G. M. (1986) *Cell* **44**, 7–19.
50. Scavarda, N. J. & Hartl, D. L. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 7515–7519.