Introduction of the Transposable Element *mariner* into the Germline of *Drosophila melanogaster*

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ABSTRACT

A chimeric *white* gene (w^{pch}) and other constructs containing the transposable element *mariner* from *Drosophila mauritiana* were introduced into the germline of *Drosophila melanogaster* using transformation mediated by the *P* element. In the absence of other *mariner* elements, the w^{pch} allele is genetically stable in both germ cells and somatic cells, indicating that the *peach* element (*i.e.*, the particular copy of *mariner* inserted in the w^{pch} allele) is inactive. However, in the presence of the active element *Mos1*, the w^{pch} allele reverts, owing to excision of the *peach* element, yielding eye-color mosaics and a high rate of germline reversion. In strains containing *Mos1* virtually every fly is an eye-color mosaic, and the rate of w^{pch} germline reversion ranges from 10 to 25%, depending on temperature. The overall rates of *mariner* excision and transposition are approximately sixfold greater than the rates in comparable strains of *Drosophila simulans*. The activity of the *Mos1* element is markedly affected by position effects at the site of *Mos1* insertion. In low level mosaic lines, dosage effects of *Mos1* are apparent in the heavier level of eye-color mosaicism in *Mos1* homozygotes than in heterozygotes. However, saturation occurs in high level mosaic lines, and then dosage effects are not observed. A pBluescribe M13+ plasmid containing *Mos1* was injected into the pole plasm of *D. melanogaster* embryos, and the *Mos1* element spontaneously integrated into the germline at high efficiency. These transformed strains of *D. melanogaster* presently contain numerous copies of *mariner* and may be useful in transposon tagging and other applications.

TRANSPOSABLE elements provide powerful tools for molecular genetics. They are able to be maintained in a variety of related genomes, serve as agents of chromosomal insertion, deletion, or rearrangement, and provide the basis for transformation of somatic or germ cells. At the same time, the biological mechanisms that control the dissemination of transposable elements among species and their persistence within species are poorly understood. The *mariner* family of transposable elements in the genus *Drosophila* is a case in point (reviewed in HARTL 1989). Elements in the *mariner* family occur in most species of the *melanogaster* species subgroup of Drosophila, but they have not yet been found in natural isolates of *D. melanogaster* itself (JACOBSON, MEDHORA and HARTL 1986). Comparisons of *mariner* sequences present in species most closely related to *D. melanogaster* (*D. simulans, D. mauritiana* and *D. sechellia*) and other, more distantly related members of the species subgroup (*D. yakuba and D. teissieri*) suggest that *mariner* invaded the genome prior to diversification of the species subgroup (MARUYAMA and HARTL 1991). The early invasion implies that the element was lost in the lineages leading to *D. melanogaster, D. erecta* and *D. orena*. On the other hand, the occurrence of highly similar mariner sequences in very distantly related species of the genus *Zaprionus* implies a relatively recent horizontal transfer (K. MARUYAMA and D. L. HARTL, unpublished).

The mariner transposable element is 1286 base pairs in length. It terminates in 28-base-pair inverted repeat sequences and contains a long open reading frame capable of coding for a polypeptide of 345 amino acids (JACOBSON, MEDHORA and HARTL 1986). The element was discovered in *D. mauritiana* through a mutant allele, *white-peach* (w^{pch}), which is genetically unstable in both somatic and germ cells (JACOBSON and HARTL 1985). Various strains of *D. mauritiana* contain 20–40 copies of *mariner*, and the w^{pch} allele has a copy of *mariner* inserted in the 5′ end of the white gene. A low level of somatic excision of the *mariner* element from w^{pch} occurs in most strains of *D. mauritiana*, yielding pigmented patches in an otherwise peach-colored eye. The infrequent occurrence of such mosaics implies that most copies of *mariner* in the genome are not highly active. Other *mariner* elements, called mosaic (*Mos*) factors, are very active. For example, in most strains, the factor *Mos1* results in eye color mosaicism in virtually every fly that carries it, and rates of germline reversion of w^{pch} in *Mos1* strains are up to 1% (BRYAN, JACOBSON and HARTL 1987).

The species distribution and genetic characteristics of *mariner* provide several strong motivations for in-

troducing the element into the genome of *D. melanogaster*. First, the presence of the element in closely related species suggests that the element will be active in the *D. melanogaster* genome, adding to the already impressive repertoire of genetic tools available in this species and providing another system for transposon tagging and perhaps germline transformation. Second, the capability for genetic manipulation in *D. melanogaster* enables detailed genetic analysis of the *mariner* element itself and host factors that may be necessary for its activity. Third, the absence of *mariner* from the normal genome of *D. melanogaster* means that genetic studies can be carried out without complications due to other *mariner* elements in the genetic background.

In this paper we report the transfer of the *mariner* system into the genome of *D. melanogaster*. A chimeric *white* gene composed mainly of *D. melanogaster* sequence, but including a *mariner*-containing restriction fragment from the w^{peach} allele of *D. mauritiana*, was introduced into the germline of *D. melanogaster* using *P* element-mediated transformation (RUBIN and SPRADLING 1982), yielding w^{peach} transformants. Transformation using the *P* element was also used to introduce the *mariner* mosaic element *Mos1* (BRYAN, JACOBSON and HARTL 1987) into the *D. melanogaster* germline. Crosses between the *D. melanogaster* w^{peach} and *Mos1* strains reconstituted the system of high level somatic mosaicism observed in *D. mauritiana*. Studies of the transformed *D. melanogaster* strains have demonstrated that the *Mos1* element is approximately eightfold more active in the genome of *D. melanogaster* than in *D. mauritiana*. However, the particular copy of *mariner* inserted in the w^{peach} allele is apparently nonfunctional. Dosage and saturation effects of *Mos1* are evident in the levels of somatic mosaicism and in the rates of germline reversion of w^{peach}, and there is a pronounced temperature effect on *Mos1* activity. In addition, the autonomous *Mos1* element is capable of spontaneous integration into the germline of *D. melanogaster*, providing the potential for the development of a general transformation system using *mariner*.

MATERIALS AND METHODS

The *white-peach* transformation vector pUChsneo:w^{peach} was constructed as illustrated in Figure 1A. First, a *P* element transformation vector containing the wild-type *white* gene from Canton S was constructed from the pUChsneo vector (STELLER and PIRROTTA 1985) by isolating a 11.7-kb *Eco*RI fragment containing *white* from plasmid p*P*[(*w*, *ry*)]A (HAZELRIGG, LEVIS and RUBIN 1984) and inserting this fragment into the *Eco*RI cloning site of pUChsneo. The pUChsneo vector was provided by V. PIRROTTA, the p*P*[(*w*, *ry*)]A plasmid by G. RUBIN. The resulting plasmid was recovered after transformation into *Escherichia coli* HB101 and was partially digested with *Bam*HI to remove the 3.0-kb *Bam*HI fragment from *white* spanning coordinates 1.0–4.0 (BINGHAM, LEVIS and RUBIN 1981). The excised *Bam*HI fragment was then replaced with the analogous *mariner*-containing 4.3-kb *Bam*HI fragment from plasmid pJJ1 (JACOBSON, MEDHORA and HARTL 1986). Clones were isolated after transformation into HB101, and the constructs having the proper orientation of the inserted *Bam*HI fragment were confirmed by restriction mapping using various combinations of *Bam*HI, *Sal*I and *Sac*I. It should be noted that pUChsneo:w^{peach} contains a chimeric white gene: the material within the 4.3 kb *Bam*HI fragment is from *D. mauritiana*, the rest of the gene is from *D. melanogaster*.

The transformation vector Car20:*Mos1* was constructed as outlined in Figure 1B. A 5.0-kb *Bam*HI-*Hin*dIII fragment containing *Mos1* (MEDHORA, MACPEEK and HARTL 1988) was isolated, the ends made flush with the Klenow fragment of DNA polymerase I, and inserted into the *Hpa*I site of the *P* element transformation vector Carnegie 20 (RUBIN and SPRADLING 1983). The orientation of the inserted fragment was determined by enzyme digestion with *Sal*I, which cleaves once at nucleotide 349 in the 1286-base-pair *mariner* element (orientation as in JACOBSON, MEDHORA and HARTL 1986). The 5.0-kb *Mos1*-containing fragment includes *Mos1* along with approximately 3.5 kb of upstream sequence and 0.2 kb of downstream sequence from *D. simulans* (MEDHORA, MARUYAMA and HARTL 1991).

The plasmid used for autonomous *mariner* transformation of the *D. melanogaster* germline was constructed by inserting the same 5.0-kb *Bam*HI-*Hin*dIII fragment containing *Mos1* into pBluescribe M13+ after digestion of the plasmid with *Bam*HI and *Hin*dIII.

Plasmid DNA was purified by CsCl-ethidium bromide equilibrium density gradient centrifugation and used for injection of embryos. Transformations were carried out essentially as described in RUBIN and SPRADLING (1982). DNA concentrations in the injection needle were approximately 150 μg/ml, and embryos were injected and incubated under oil at 18°. Emerging larvae were transferred to standard Drosophila medium and maintained at 25°. In the transformations with *P* element we used the wings-clipped helper plasmid pπ25.7WC (KARESS and RUBIN 1984) at a concentration of approximately 75 μg/ml.

General procedures for DNA isolation, enzyme digestion, electrophoresis and blotting are described in BRYAN, GARZA and HARTL (1990). General procedures for recombinant DNA manipulation are described in MANIATIS, FRITSCH and SAMBROOK (1982).

RESULTS

Transformation of w^{peach} and *Mos1*: *D. melanogaster* embryos of genotype *y* w^{67c23} (chosen because it is a partial deletion of *white*) were injected with the chimeric w^{peach} gene contained in the *P* element vector pUChsneo:w^{peach}, diagrammed in Figure 1A, and coinjected with the wings-clipped helper plasmid (KARESS and RUBIN 1984). Among 138 surviving G_0 offspring, 108 were fertile. One of these gave offspring with peach-like eye color, and these were individually crossed with the *y* w^{67c23} stock for further analysis. Genetic studies indicated two independent insertion events had occurred in the transformed G_0. Two of the *white-peach* offspring carried insertions into an identical position in the *X* chromosome (designated sublines P735 and P739), and the other *white-peach* offspring had an insertion into chromosome *3* (subline P734). The insertion into chromosome *3* is associated

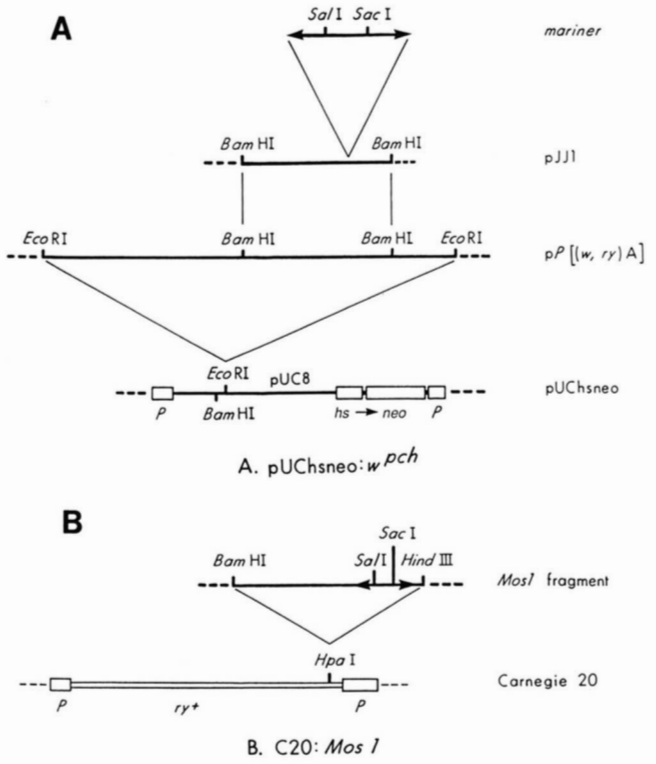

FIGURE 1.—Transformation vectors for the introduction of *mariner* into *D. melanogaster*. (A) The w^{pch} vector was created by replacing the indicated *Bam*HI fragment in the *D. melanogaster white* gene with the corresponding fragment from the w^{pch} allele from *D. mauritiana*. This fragment contains the *mariner* element designated *peach*. (B) The *Mos1* vector was created by inserting a *Mos1*-containing *Bam*HI-*Hin*dIII fragment into the *Hpa*I cloning site in Carnegie 20.

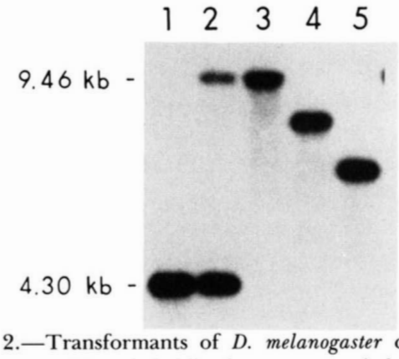

FIGURE 2.—Transformants of *D. melanogaster* containing the *mariner* element. Filter hybridizations were carried out with DNA from various strains digested with *Bam*HI and probed with *mariner* DNA. The lanes are as follows: (1) strain P735, which contains an insertion of w^{pch}; (2) P735 insertion in combination with a single insertion of *Mos1*; (3)–(5) single insertions of *Mos1* at different positions in the genome, in the absence of w^{pch}.

with a recessive lethal, which has thus far been inseparable from the site of the insertion.

None of the transformed lines give any evidence of somatic or germinal instability of w^{pch}. Somatic instability is indicated by excision events that give occasional pigmented facets in the eye, and germinal instability is indicated by reverse mutation of w^{pch} to w^{+} or forward mutation to w^{-}. The results with *D. melanogaster* are in marked contrast to those with *D. mauritiana* or *D. simulans*, in which w^{pch} always gives some detectable level of somatic excision (JACOBSON and HARTL 1985; HAYMER and MARSH 1986). The difference between the species suggests that the *mariner* element inserted in w^{pch} is inactive and that the instability of w^{pch} in the other species results from *trans*-activation by other copies of *mariner* in the genetic background. We therefore designate the particular *mariner* element present in w^{pch} as the *peach* element. The sequence of this element has been determined (JACOBSON, MEDHORA and HARTL 1986). It is possible that the *peach* element in *D. melanogaster* might have undergone mutations in the course of subcloning and *in vitro* manipulation. However, the conclusion that the *peach* element is inactive is supported by genetic experiments in a strain of *D. simulans* that contains no other copies of *mariner* in the genetic background. When the w^{pch} allele was introduced into this strain by repeated backcrossing, and all other copies of *mariner* in the genetic background were eliminated by further backcrossing, the resulting w^{pch} strains were somatically and germinally stable (G. J. BRYAN and D. L. HARTL, unpublished), as observed in the *D. melanogaster* w^{pch} strains.

Transformants of *D. melanogaster* carrying *Mos1* were obtained using the vector C20:*Mos1* diagrammed in Figure 1B, which was injected along with the wings-clipped helper into the strain ry^{506}. Survival of injected embryos to eclosion was approximately 5–10%. About 80% of the eclosing G_0 individuals were fertile, and germline transformants were obtained from 13% of the fertile G_0. Most of the transformed lines contained a single copy of the $P[ry^{+}\ Mos1]$ transposon (Figure 2). Three transformed lines bearing single insertions–M3, M16 and R8–were chosen for further study. Lines M3 and M16 have independent insertions of $P[ry^{+}\ Mos1]$ into chromosome *2*, and line R8 has an insertion into chromosome *3*. All three lines are homozygous viable.

Interactions of *Mos1* with w^{pch}: The *X*-linked and third-chromosomal w^{pch} lines, when combined with any of the *Mos1* lines M3, M16 or R8, yield somatic mosaics in which the eye-color phenotype of each fly is a mixture of red pigmented and peach-colored facets. This result demonstrates that the *Mos1* factor functions in the genome of *D. melanogaster* and that it continues to recognize the *peach* element as a target for excision. In Figure 3, the band at 4.3 kb in lane 1 is a *Bam*HI fragment containing upstream sequences from *white* along with the *peach* element. The band at 3.0 kb in lane 2 results from somatic excision of *peach* in flies bearing the M3 *Mos1* insertion. The *Mos1* element is also associated with a maternal effect, unique among transposable elements, in which factors transmitted through the egg result in somatic excision in the progeny (BRYAN and HARTL 1988). That is,

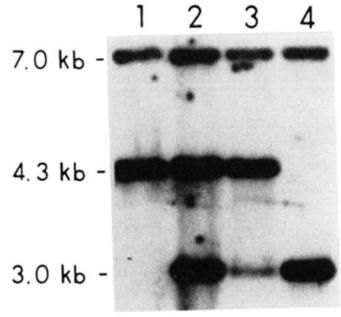

FIGURE 3.—Filter hybridizations of *D. melanogaster* DNA digested with *Bam*HI and probed with a 3.0-kb *Bam*HI fragment of *white* DNA. The 7.0-kb fragment is characteristic of the w^{67c23} deletion, which is in the common genetic background of the strains. The 3.0-kb fragment is derived from the wildtype gene (in both *D. melanogaster* and *D. mauritiana*), and the 4.3-kb fragment is derived from the *D. mauritiana* w^{pch} fragment containing the peach element. In each case the w^{pch} insertion is that in strain P735. Lanes are as follows: (1) w^{67c23}, w^{pch}; (2) w^{67c23}, w^{pch}; *Mos1* (M3 insertion); (3) w^{67c23}, w^{pch} maternal-effect mosaic; (4) w^{67c23}, w^{+} revertant.

females that are heterozygous for *Mos1* have predominantly mosaic offspring, even though half of the progeny lack *Mos1*. In most stocks the maternal-effect mosaics can be identified by phenotype because they show very few reversion events that occur early in development, resulting in one (occasionally more) large sectors of pigmented facets, whereas their *Mos1*/+ siblings have frequent reversion events occurring at different stages of eye development resulting in a more speckled phenotype (*e.g.*, Figure 1 in BRYAN and HARTL 1988). DNA from maternal-effect mosaics of *D. melanogaster* is shown in lane 3 in Figure 3. The occurrence of somatic excision of *peach* in these individuals is clear, although the excision occurs to a lesser extent than in sibs carrying the *P*[ry^{+} *Mos1*] transposon.

The character of the eye-color mosaicism differs somewhat among the M3, M16 and R8 lines. In combination with w^{pch}, the *Mos1* line M3 gives heavy mosaicism (defined as large, overlapping patches of pigmented tissue), line M16 gives light mosaicism (defined as small, scattered patches of pigmented tissue), and line R8 gives an intermediate level of mosaicism.

One straightforward explanation of the different levels of mosaicism in the *Mos1* lines is that *Mos1* expression is affected by position effects resulting from genomic sequences at or near the site of insertion. This interpretation is supported by additional genetic experiments, described in the next section, in which the *Mos1* factor had undergone transposition to still other positions in the genome.

Position effects on *Mos1* expression: Mobilization of the *Mos1* transposon can occur either autonomously or as a result of mobilization by the modified *P* element *P*[ry^{+} *Δ2-3*](*99B*), which produces high levels of *P* transposase activity but low levels of transposition and excision (ROBERTSON *et al.* 1988). Various types of crosses were carried out to obtain *Mos1* factors in different positions in the genome.

For the *Mos1* insertions in chromosome *2*, males of genotype *P*[ry^{+} *Mos1*]/*CyO*, *Cy*; ry^{506} *Sb P*[ry^{+} *Δ2-3*](*99B*)/*ry* were crossed with *ry* females, and the nonrosy, nonstubble, *CyO*/+ offspring were crossed with w^{pch} in order to detect eye-color mosaicism. Among 15 transpositions obtained from the M3 and M16 *Mos1* lines in this manner, eight had the *P*[ry^{+} *Mos1*] transposon located in the *CyO* balancer and seven were in the third chromosome. In an analogous scheme using the R8 insertion into chromosome *3*, *CyO*, *Cy*/*Sp*; ry^{506} *Sb P*[ry^{+} *Δ2-3*](*99B*)/*P*[ry^{+} *Mos1*] flies were crossed with *ry*, and the nonrosy, stubble offspring were crossed with w^{pch} in order to detect eye-color mosaicism. Among 12 transpositions detected in this manner, three were in the *X* chromosome, two in chromosome *2*, and seven in chromosome *3*. In the absence of *P*[ry^{+} *Δ2-3*](*99B*), among 12 autonomous transpositions of the *Mos1* factor in the M3 strain, three transpositions were into the *X* chromosome and nine into chromosome *3*. As expected, these transposed *Mos1* elements were no longer associated with ry^{+}.

Altogether we examined 39 lines with transpositions of either the *P*[ry^{+} *Mos1*] transposon or of *Mos1* itself. The degree of mosaicism among these lines varied markedly, from one extreme of a very light speckle of pigmented facets to the other extreme of extremely heavy mosaicism with almost fully pigmented eyes. That a wide range of mosaic types can result from transpositions of the identical *Mos1* factor implies that much of the variation in phenotype among the lines is attributable to position effects on expression of *Mos1*. Marked variation in level of mosaicism was also observed in the transpositions bearing the *P*[ry^{+} *Mos1*] transposon. Insertions of this element contain approximately 11 kb of ry^{+} and *D. simulans* DNA separating the *Mos1* element from genomic sequences upstream of the insertion site (Figure 1B), indicating that the position effects may extend over substantial distances. On the other hand, there are approximately 200 base pairs of flanking DNA on the downstream side of the *P*[ry^{+} *Mos1*] transposon, and perhaps some of the position effects are mediated from this end.

Dosage and saturation effects: In addition to position effects on *Mos1* expression are clear effects of gene dosage. For comparative purposes, rates of germline reversion are preferable to subjective assessments of degree of somatic mosaicism because they are more quantitative. Reversion data are summarized for the M16 (light mosaic) and M3 (heavy mosaic) lines in Table 1. The numbers tabulated are the frequencies of phenotypically w^{+} offspring resulting

TABLE 1

Reversion rates of w^{pch} allele

Genotype	M16 (light mosaic)	M3 (heavy mosaic)
w^{pch}/Y; *Mos1/CyO* ♂♂	11.6 ± 0.6 (2742)	15.6 ± 0.9 (1640)
w^{pch}/Y; *Mos1/Mos1* ♂♂	24.0 ± 2.2 (379)	17.4 ± 1.4 (703)
w^{pch}/w^{pch}; *Mos1/CyO* ♀♀	9.9 ± 0.5 (3475)	15.3 ± 0.6 (3453)
w^{pch}/w^{pch}; *Mos1/Mos1* ♀♀	ND[a]	15.7 ± 1.3 (795)

Tabulated values are percentages. The numbers in parentheses are the total numbers of progeny.

[a] Not determined.

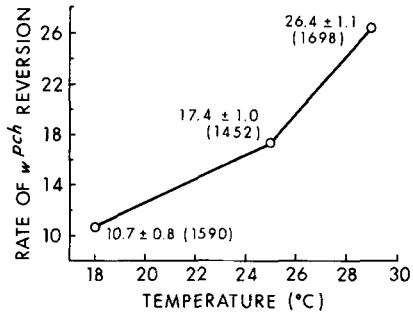

FIGURE 4.—Rate of reversion of w^{pch} to phenotypic w^+ as a function of temperature in the *Mos1* strain M3. Rates are given as percentages.

from reversion of w^{pch} to w^+ in various genotypes. Although the revertants are phenotypically wild type, DNA sequencing in *D. mauritiana* indicates that the reversion events are usually somewhat imprecise (BRYAN, GARZA and HARTL 1990). The frequencies of reversion were estimated by the proportion of wild-type progeny, since there was no significant tendency for wild-type progeny to occur in clusters.

Overall, the rate of w^{pch} reversion in *D. melanogaster* is much greater than in *D. mauritiana* . The average rate of reversion among homozygous *Mos1* males from the M16 and M3 lines is approximately 20%, which is about eightfold greater than the rate of reversion in homozygous *Mos1* males in the E25H line in *D. mauritiana* (BRYAN, JACOBSON and HARTL 1987). Moreover, there is virtually no difference in the rate of w^{pch} reversion in males and females in *D. melanogaster* ($P = 0.15$), whereas in *D. mauritiana* there is a 2.5-fold greater rate of reversion in males (JACOBSON and HARTL 1985).

As noted, the degree of somatic mosaicism is markedly less in the M16 line than in the M3 line. A significant difference is also reflected in the rate of germline reversion, which in heterozygous *Mos1* genotypes is 34% greater in males and 54% greater in females (P values in χ^2 tests are both less than 0.001).

Dosage effects of *Mos1* are apparent in the M16 line, in which the homozygotes have an approximately twofold greater rate of reversion than the heterozygotes. The differences are also clear in the degree of somatic mosaicism: *Mos1* homozygotes in the M16 line have larger pigmented patches than the heterozygotes, and the genotypes can usually be distinguished on this basis. However, a difference between heterozygotes and homozygotes is not seen in the heavy mosaic line M3. The M3 *Mos1* heterozygotes and homozygotes cannot be distinguished by degree of somatic mosaicism, and the rate of w^{pch} reversion in the homozygotes is only slightly (and not significantly) different from what it is in the heterozygotes ($P = 0.6$ in a χ^2 test). The absence of a dosage effect with M3 suggests a saturation effect in which the amount of *Mos1* gene product is no longer limiting to the rate of peach excision.

Temperature effects: We have also observed a pronounced temperature effect on *Mos1* expression. The effect is qualitatively evident in the extent of eye color mosaicism, which is considerably lighter at 18° than at 25°. Quantitative comparisons can be based on rates of germline excision of the *peach* element. Males carrying w^{pch} and heterozygous for a *Mos1* chromosome from the M3 line were raised at 18°, 25° or 29° and crossed with attached-*X* females at 25° to estimate the proportions of w^{pch} and phenotypically w^+ male progeny. The data are summarized in Figure 4. The differences between the temperatures are highly significant. It is extraordinary that, at 29°, more than 25% of all the *X* chromosomes undergo excision of the *peach* element. A similar experiment with *Mos1*/+ *D. simulans* males mated with attached-*X* females gave the following result: w^{pch} excision at 18°, 0.2% ± 0.1% ($N = 1304$); w^{pch} excision at 25°, 3.4% ± 0.5% ($N = 1266$). As compared with *D. simulans*, the rate of w^{pch} excision in *D. melanogaster* is about five times greater at 25° and about 50 times greater at 18°. The particular *D. simulans* strain tested is designated Z9, which is a strain carrying w^{pch} and *Mos1* that had been backcrossed repeatedly to a *mariner*-free strain of *D. simulans* and at the time of testing had few or no copies of *mariner* beyond *peach* and *Mos1* (G. J. BRYAN and D. L. HARTL, unpublished).

Excision and transposition of *Mos1*: The *Mos1* element promotes not only the excision of *peach* but also its own excision and transposition (MEDHORA, MACPEEK and HARTL 1988). Rates of these events in *D. melanogaster* are summarized in Table 2. Since the mosaicism is completely penetrant in *Mos1* flies, the excision of *Mos1* can be assayed by the occurrence of exceptional *Curly*, nonmosaic offspring among the progeny of *Mos1/CyO* flies. In contrast to the situation with w^{pch} excision, where each event appears to occur independently, in the excision and transposition of *Mos1* there is some tendency for exceptional offspring to occur in clusters. Therefore the rates were estimated conservatively using the LURIA and DELBRUCK (1943) method. A series of single pair matings was established in vials, and the rate of excision was estimated from the proportion P_0 of vials containing no

TABLE 2

Excision and transposition rates of *Mos1*

Genotype	M16 (light mosaic)	M3 (heavy mosaic)
Excision (non-*Cy* nonmosaics)		
w^{pch}/Y; *Mos1/CyO* ♂♂ (41)	1.9 ± 0.4 (70.3)	1.0 ± 0.2 (55.5)
w^{pch}/w^{pch}; *Mos1/CyO* ♀♀ (52)	1.3 ± 0.3 (33.9)	2.0 ± 0.4 (26.3)
Transposition (*Cy* mosaics)		
w^{pch}/Y; *Mos1/CyO* ♂♂ (32)	2.4 ± 0.5 (56.8)	1.0 ± 0.3 (37.7)
w^{pch}/w^{pch}; *Mos1/CyO* ♀♀ (53)	3.8 ± 0.7 (29.6)	2.2 ± 0.4 (24.8)

Tabulated values are percentages. The numbers in parentheses after the genotypes are the numbers of matings; those in parentheses after the standard errors are the (harmonic) mean numbers of progeny. In each case the rate of excision was estimated from the proportion P_0 of vials containing no exceptional offspring as rate = $-(1/N)ln(P_0)$, where N is the average number of offspring of the relevant genotype per vial. The approximate standard errors were estimated by the asymptotic delta method as $(1/N)^2(1/P_0)^2Var(P_0)$, where $Var(P_0)$ is binomial.

exceptional offspring as $rate = -(1/N)\ln(P_0)$, where N is the average number of offspring of the relevant genotype (*e.g.*, *CyO*) per vial. The number of vials tested is given in parenthesis after each genotype, and the average number of offspring of the relevant genotype is given in parenthesis after the standard errors.

The estimated rates of *Mos1* excision are given in the upper part of Table 2. The rates are much lower than those of *peach* excision, and there is virtually no difference between the M3 and M16 lines in spite of their obviously different levels of eye-color mosaicism.

A similar result was found with respect to *Mos1* transposition, summarized in the lower part of Table 2. Transposition events were assayed by the occurrence of exceptional *Curly* mosaic progeny among the progeny of *Mos1/CyO* flies, and the rates were estimated as described above. By the nature of the mating scheme, any transpositions of *Mos1* to new locations along the same chromosome are undetectable. In the M16 line the rate of detectable transposition of *Mos1* appears to be somewhat greater than the rate of excision, but in the M16 line the rates are comparable.

Germline transformation with mariner: We have also investigated the capability of the *Mos1* element to insert autonomously into the genome when injected into the pole plasm of Drosophila embryos, using a vector with the 5.0-kb *Bam*HI-*Hin*dIII fragment containing *Mos1* inserted into the cloning site of pBluescribe M13+. Embryos of a *cn; ry* strain obtained from P. M. BINGHAM were injected. Surviving G_0 flies were crossed individually with w^{pch} *D. melanogaster* and the progeny examined visually for eye-color mosaicism. Groups of G_1 progeny were sib mated and their offspring examined for mosaicism in the G_2 and G_3 generations. Among 150 injected *cn; ry* embryos, 28 hatched, 19 survived to eclosion and 16 were fertile. Five of the 16 fertile G_0 adults yielded at least one

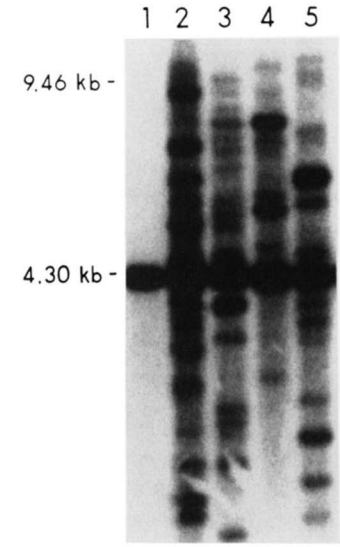

FIGURE 5.—Accumulation of *mariner* elements in spontaneously transformed lines of *D. melanogaster* containing *Mos1*. In each case the genetic background contains the w^{pch} insertion P735. Genomic DNA was digested with *Bam*HI and *Hin*dIII and probed with an *Ssp*I-*Nhe*I fragment of *mariner*. Lanes are as follows: (1) w^{67c23}, w^{pch}; (2)–(5) independent lines transformed with *Mos1*, designated A, D, H and X, respectively.

mosaic offspring from which a *Mos1*-bearing strain was established by backcrossing to w^{pch} and sib mating.

A few generations subsequent to the establishment of the transformed *Mos1* lines, chromosomal DNA was prepared and digested with *Bam*HI and *Hin*dII and probed with a 1-kb internal *Ssp*I-*Nhe*I fragment of *mariner* deriving from coordinates 56–1183 (JACOBSON, MEDHORA and HARTL 1986). Multiple *mariner*-containing bands were observed in the transformed lines, indicating that the integrated *Mos1* element was promoting active transposition and accumulation of copies (Figure 5).

DISCUSSION

The system of inherited eye-color mosaicism resulting from excision of the transposable element *mariner* from the w^{pch} allele has been transferred into the genome of *D. melanogaster*, a species in which *mariner* does not normally occur (JACOBSON, MEDHORA and HARTL 1986). The particular w^{pch} allele transformed into *D. melanogaster* is a chimeric gene consisting of the *D. melanogaster* w^+ sequence into which has been substituted a 4.3-kb *Bam*HI fragment derived from coordinates 1.0–4.0 of the *D. mauritiana* w^+ gene that contains the particular *mariner* element designated *peach* (coordinates defined as in JACOBSON, MEDHORA and HARTL 1986). The *peach* element is inactive, and so, in the absence of other *mariner* elements, the chimeric w^{pch} allele present in strains of *D. melanogaster* is genetically stable in both somatic and germ cells. However, in the presence of the active *mariner* element *Mos1*, excision of the *peach* element occurs,

resulting eye-color mosaicism and a rate of germline reversion of 10–25% or more, depending on temperature.

One noteworthy feature of Table 2 is that the rate of transposition of *Mos1*, relative to the rate of excision, is substantially greater than observed among other Drosophila transposons (see reviews in BERG and HOWE 1989). In the M16 line, the rate of transposition of *Mos1* may actually exceed the rate of excision; and in the M3 line, the rates of transposition and excision are approximately equal. Such a high rate of *Mos1* transposition, relative to the rate of excision, should favor the persistence of *Mos1* elements in the genome. Consistent with this suggestion, CAPY *et al.* (1990) have found that active *mariner* transposable elements are widespread in natural populations of *D. simulans*.

The presence of *mariner* in *D. melanogaster* provides a new means of genetic manipulation in this species that may be advantageous for certain purposes. Since the *mariner* system has been used for transposon tagging of genes in *D. mauritiana* (BRYAN, GARZA and HARTL 1990), it may be useful for this purpose in *D. melanogaster* as well. Since *mariner* has a different target-site specificity than the *P* element (O'HARE and RUBIN 1983; BRYAN, GARZA and HARTL 1990), genes that are poor targets for one element may be better targets for the other. One potentially useful feature of the *mariner* system is that new mutations resulting from *mariner* insertions can be identified by their high rate of somatic and germline excision in the presence of *Mos1*. Novel applications might include the analysis of developmental mutations induced by *mariner* insertions, since somatic excision will yield large numbers of somatic mosaics, and various temperatures can be used to control the rate and also perhaps the timing of the excision events. The high rate of somatic excision could also be used to identify nonautonomous *mariner* insertions that have mutant phenotypes only in the absence of *Mos1*-induced excision.

The finding that *Mos1* itself can efficiently insert into the *D. melanogaster* genome also suggests the possibility of developing an alternative system for germline transformation, toward which the construction of suitable cloning vectors and preliminary tests are presently underway. The *mariner* element contains a single, long, uninterrupted open reading frame (JACOBSON, MEDHORA and HARTL 1986), and the element may be able to function in a wide variety of genomes. In this connection it is interesting to note the presence of a *mariner*-like element in the genome of the Cecropia moth *Hyalophora cecropia*. The particular copy of the Cecropia element that has been sequenced is 1251 base pairs in length and contains a protein-coding region that has 55% similarity in amino acid composition with the putative coding region of *mariner* from *D. mauritiana* (LIDHOLM, GUDMUNDSSON and BOMAN, personal communication). However, the presence of several frameshift differences in the Cecropia element suggests that it is nonfunctional. There are approximately 2000 copies of these *mariner*-like elements in the Cecropia genome. The presence of *mariner*-like elements in this Lepidopteran suggests that a successful transformation system based on *mariner* might have a broad host range.

Presence of the *mariner* element in *D. melanogaster* opens up new experimental approaches to the study of transposable element function. For example, different *Mos* factors and chimeric constructs can be studied in *D. melanogaster* using the w^{pch} allele (MEDHORA, MARUYAMA and HARTL 1991), and dosage effects of *Mos* factors and *mariner* target sequences can be determined. The complete penetrance of eye-color mosaicism in w^{pch}; *Mos1* flies also provides easy phenotypic detection of mutations that eliminate the excision of w^{pch}. Such mutations in the *peach* element will affect the ability of the element to serve as a target or to excise, and those in *Mos1* will affect expression or activity of the element. However, the system should also identify mutations in chromosomal genes that are necessary for *mariner* target recognition or excision. It should also be noted that the w^{pch}-*Mos1* system can serve as an enhancer trap for insertions of *Mos1* near enhancers that stimulate differential expression across the eye. During routine stockkeeping a mosaic strain of *D. simulans* has been isolated in which the mosaic patches occur primarily in the anterior portion of the eye (BRYAN 1989).

The rate of germline excision of the *peach* element in *Mos1*-bearing strains of *D. melanogaster* is approximately 17% at 25°, as compared with approximately 3% in *D. simulans*–about a sixfold difference. Rates of *Mos1* excision and transposition are not as high as that of *peach* excision, but they are still substantially greater than in *D. simulans* (MEDHORA, MACPEEK and HARTL 1988). We do not know why the rates of excision and transposition are so much greater in *D. melanogaster* than in *D. simulans*. Since strains of *D. simulans* containing only w^{pch} and *Mos1* do not have rates of *peach* excision comparable to those in *D. melanogaster* (G. J. BRYAN and D. L. HARTL, unpublished), the difference is not likely to result merely from other copies of *mariner* in the genetic background. A more likely possibility is that the species differ because of host factors with which *mariner* or its products interact–and many host factors must be involved in the transcription, translation, transport, and function of the *mariner* product. It is even possible that the different *mariner* behavior in the two species reflects a more general process, not yet understood, by which, on average, the abundance of transposable

elements in *D. melanogaster* is some sevenfold greater than in *D. simulans* or *D. mauritiana* (DOWSETT and YOUNG 1982).

It is also unclear why the rate of *peach* excision is so much greater than that of *Mos1*, but the likely candidates are differences in recognition sequences in the elements themselves (MEDHORA, MARUYAMA and HARTL 1991) or differences in their accessibility in the genome. Whatever the mechanism, the result is that the level of eye-color mosaicism is not necessarily correlated with the rate of *Mos1* excision or transposition.

Transposable elements are capable of undergoing horizontal transfer between species. The population dynamics of *P* element in a new host genome has been studied by experimental transfer of this element into *D. simulans* (SCAVARDA and HARTL 1984, 1987; DANIELS, STRAUSBAUGH and ARMSTRONG 1985; DANIELS, CHOVNICK and KIDWELL 1989). The introduction of *mariner* into *D. melanogaster* provides experimental opportunities that are in some ways superior because of the repertoire of mutants and chromosome rearrangements that are available in *D. melanogaster*. The *Mos1*-transformed strains of *D. melanogaster* presently contain numerous copies of *mariner* at many scattered positions throughout the genome. It will be of some interest to determine whether the *D. melanogaster* populations evolve toward a state comparable to that in many widely distributed natural populations of *D. simulans* that contain active *Mos*-like elements (CAPY *et al.* 1990).

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LITERATURE CITED

BERG, D. E., and M. M. HOWE (Editors), 1989 *Mobile DNA*. American Society for Microbiology, Washington, D.C.

BINGHAM, P. M., R. LEVIS and G. M. RUBIN, 1981 Cloning of DNA sequences from the *white* locus of *D. melanogaster* by a novel and general method. Cell **25:** 693–704.

BRYAN, G. J. , 1989 Molecular analysis of transposon-induced mutations and their derivatives in Drosophila. Ph.D. thesis, Washington University, St. Louis.

BRYAN, G. J., D. GARZA and D. L. HARTL, 1990 Insertion and excision of the transposable element *mariner* in Drosophila. Genetics **125:** 103–114.

BRYAN, G. J., and D. L. HARTL, 1988 Maternally inherited transposon excision in *Drosophila simulans*. Science **240:** 215–217.

BRYAN, G. J., J. W. JACOBSON and D. L. HARTL, 1987 Heritable somatic excision of a *Drosophila* transposon. Science **235:** 1636–1638.

CAPY, P., F. CHAKRANI, F. LEMEUNIER, D. L. HARTL and J. R. DAVID, 1990 Active *mariner* transposable elements are widespread in natural populations of *Drosophila simulans*. Proc. Roy. Soc. Lond. B **242:** 57–60.

DANIELS, S. B., A. CHOVNICK and M. G. KIDWELL, 1989 Hybrid dysgenesis in *Drosophila simulans* lines transformed with autonomous *P* elements. Genetics **121:** 281–291.

DANIELS, S. B., L. D. STRAUSBAUGH and R. A. ARMSTRONG, 1985 Molecular analysis of *P* element behavior in *Drosophila simulans*. Mol. Gen. Genet. **200:** 258–265.

DOWSETT, A. P., and M. W. YOUNG, 1982 Differing levels of dispersed repetitive DNA among closely related species of Drosophila. Proc. Natl. Acad. Sci. USA **79:** 4570–4574.

HARTL, D. L. , 1989 Transposable element *mariner* in Drosophila species, pp. 531–536 in *Mobile DNA*, edited by D. E. BERG and M. HOWE. American Society for Microbiology, Washington, D.C.

HAYMER, D. S., and J. L. MARSH, 1986 Germ line and somatic instability of a *white* mutation in *Drosophila mauritiana* due to a transposable element. Dev. Genet. **6:** 281–291.

HAZELRIGG, T., R. LEVIS and G. M. RUBIN, 1984 Transformation of *white* locus DNA in Drosophila: dosage compensation, *zeste* interaction, and position effects. Cell **36:** 469–481.

JACOBSON, J. W., and D. L. HARTL, 1985 Coupled instability of two *X*-linked genes in *Drosophila mauritiana:* Germinal and somatic mutability. Genetics **111:** 57–65.

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

KARESS, R. E., and G. M. RUBIN, 1984 Analysis of transposable element functions in Drosophila. Cell **38:** 135–146.

LURIA, S., and M. DELBRUCK, 1943 Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28:** 491–511.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

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

MEDHORA, M. M., A. H. MACPEEK and D. L. HARTL, 1988 Excision of the *Drosophila* transposable element *mariner*: indentification and characterization of the *Mos* factor. EMBO J. **7:** 2185–2189.

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

O'HARE, K., and G. M. RUBIN, 1983 Structure of *P* transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. Cell **34:** 25–35.

ROBERTSON, H. M., C. R. PRESTON, R. W. PHILLIS, D. JOHNSON-SCHLITZ, W. K. BENZ and W. R. ENGELS, 1988 A stable genomic source of *P* element transposase in *Drosophila melanogaster*. Genetics **119:** 75–83.

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

RUBIN, G. M., and A. C. SPRADLING, 1983 Vectors for *P* element-mediated gene transfer in Drosophila. Nucleic Acids Res. **11:** 6341–6351.

SCAVARDA, N. J., and D. L. HARTL, 1984 Interspecific DNA transformation in Drosophila. Proc. Natl. Acad. Sci. USA **81:** 7515–7519.

SCAVARDA, N. J., and D. L. HARTL, 1987 Germline abnormalities in *Drosophila simulans* transfected with the transposable *P* element. J. Genet. **66:** 1–15.

STELLER, H., and V. PIRROTTA, 1985 A transposable *P* vector that confers selectable G418 resistance to Drosophila larvae. EMBO J. **4:** 167–171.

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