Genotypic Effects, Maternal Effects and Grand-Maternal Effects of Immobilized Derivatives of the Transposable Element *mariner*

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ABSTRACT

The baseline rate of spontaneous integration of the autonomous *mariner* element *Mos1* into the germline of *Drosophila melanogaster* is estimated as 16 ± 5% (mean ± SE) among fertile G0 flies. However, the transformation rate is reduced ~20-fold in *Mos1* constructs with exogenous DNA in the size range 5–12 kb inserted into the *Sad* site. To provide alternative *Mos1* helper plasmids for transformation experiments, two types of *Mos1*-promoter fusions were constructed: *hsp-70:Mos1* and *hsp26-Sgs3:Mos1*. The former has the *Mos1* coding region driven by the *hsp70* heat-shock promoter; the latter has it driven by the basal *Sgs3* promoter under the control of the *hsp26* female-germline specific transcriptional regulator. When introduced into *D. melanogaster* by *P*-element-mediated germline transformation, these elements are unable to transpose or excise in the presence of autonomous *Mos1*-related elements (they are "marooned") because the 5′ inverted repeat of *Mos1* is missing. As expected, the *hsp26-Sgs3:Mos1* fusions exhibit a significantly greater rate of germline excision of a target *mariner* element than do the *hsp70:Mos1* fusions. Unexpectedly, the rate of excision of target *mariner* elements induced by *hsp26-Sgs3:Mos1* is the same in the male germline as in the female germline. Both *hsp:Mos1* fusions show strong germline expression and a maternal effect of the *mariner* transposase. A significant grand-maternal effect of the *hsp:Mos1* fusions was also detected as a result of a maternal effect on the germline of the F_1 progeny. Among flies carrying the promoter fusions inherited maternally, about three-quarters of the overall rate of germline excision derives from the direct genotypic effect and about one-quarter results from the grand-maternal effect. Despite the strong somatic expression of the *hsp:Mos1* fusions, *mariner* transformants carrying a *white*$^+$ reporter gene at the *Sad* site remained stable in the soma.

THE transposable element *mariner* has considerable potential as a vector for the genetic manipulation of insect agricultural pests and carriers of human disease (KIDWELL 1993). The element has been shown to integrate spontaneously into the germline of *Drosophila melanogaster* (GARZA *et al.* 1991), and its ability to mediate germline transformation with exogenous DNA has been demonstrated (LIDHOLM *et al.* 1993). Moreover, *mariner*-like elements (MLEs) are widely distributed among insects and other invertebrates (LIDHOLM *et al.* 1991; ROBERTSON 1993; ROBERTSON and MACLEOD 1993; CAPY *et al.* 1994; LOHE *et al.* 1995). Among 404 insect species examined for the presence of MLEs, 63 species (15.6%) were able to support DNA amplification with oligonucleotide primers to conserved regions (ROBERTSON and MACLEOD 1993). The MLEs can be classified into several distinct subfamilies according to similarities in nucleotide sequence. MLEs in different subfamilies are typically 40–56% identical in nucleotide sequence (ROBERTSON and MACLEOD 1993). The major subfamilies all appear to be widely distributed among species, and any particular species may contain MLEs from two or more different subfamilies (ROBERTSON 1993; LOHE *et al.* 1995). The genetic diversity among MLEs suggests an ancient lineage undergoing progressive sequence diversification. The widespread distribution of MLEs among species is apparently the result of horizontal transmission among species (MARUYAMA and HARTL 1991a; ROBERTSON 1993; ROBERTSON and MACLEOD 1993; CAPY *et al.* 1994; LOHE *et al.* 1995) balanced against factors leading to loss of the element ("stochastic loss") as well as natural selection acting to minimize the harmful mutagenic effects of transposition ("vertical inactivation"; LOHE *et al.* 1995).

Among the diversity of MLEs found in invertebrate genomes, the vast majority are apparently nonfunctional owing to the presence of one or more chain-terminating or frameshift mutations that destroy the open reading frame (ROBERTSON and MACLEOD 1993; CAPY *et al.* 1994; LOHE *et al.* 1995). The functional elements so far identified are all closely related to the active *Mos1* element isolated from *D. mauritiana* (MARUYAMA *et al.* 1991; MEDHORA *et al.* 1991; CAPY *et al.* 1992). The *Mos1* element is active in transposition (MEDHORA *et al.* 1991), capable of *trans* complementation of inactive (nonautonomous) elements (GARZA *et al.* 1991), and able to mediate germline transformation with exogenous DNA (LIDHOLM *et al.* 1993).

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Unlike a few well-characterized Drosophila transformation systems based on other transposable elements, the parameters influencing the mobilization of *mariner* elements containing exogenous DNA and governing the efficiency of *mariner*-mediated transformation have not been fully defined. Genetic analysis in these other systems has been greatly facilitated by the use of strains of flies containing a genetically stable source of transposase—the *P*[*ry*$^+$ Δ2–3](99B) insertion (ROBERTSON *et al.* 1988) and the Icarus construct (STELLER and PIRROTTA 1986) in the case of *P* element as well as the *P*[*ry*$^+$, HSH2] construct in the case of *hobo* (CALVI and GELBART 1994). Both Icarus and *P*[*ry*$^+$, HSH2] contain protein-coding sequences driven by the heat-shock promoter *hsp70* (STELLER and PIRROTTA 1984). In this report, we describe the construction, germline transformation, and genetic analysis of derivatives of the *Mos1* element in which the open reading frame is driven by either the *hsp70* promoter or the *hsp26-Sgs3* chimeric promoter reported to be specific for the female germline (FRANK *et al.* 1992). These elements lack the 5′ inverted repeat of *Mos1* and are unable to transpose, hence they are said to be "marooned." In the course of these experiments, we have also demonstrated germline transformation with a *Mos1* derivative containing the mini-*white* gene (PIRROTTA *et al.* 1985), and we have estimated that the rate of spontaneous integration of *Mos1* into the germline of *D. melanogaster* as ~16 ± 5%.

MATERIALS AND METHODS

Construction of pM789 and pM789[mini-*white*] vectors: General procedures for recombinant DNA manipulation are described in SAMBROOK *et al.* (1989). Synthesis of a *Mos1* transformation vector containing the mini-*white* gene made use of the plasmids pA2L and pB2R described in LIDHOLM *et al.* (1993). The plasmid pA2L contains a 1.2kb *Sac*I fragment that includes 0.4 kb of *D. simulans* DNA flanking the 5′ part of *Mos1* and extending from the 5′ inverted repeat through the *Sac*I site at position 789; this fragment was blunt-end ligated into the *Sma*I site in the polylinker of pBluescript SK (Stratagene). The plasmid pB2R contains a 0.7-kb *Sac*I-*Hin*dIII fragment that includes the 3′ part of *Mos1* from the *Sac*I site from position 790 through and into the 0.2 kb of flanking *D. simulans* DNA; this fragment was ligated into the *Hinc*II-*Hin*dIII site in the polylinker in pBluescript KS (the *Sac*I-*Hin*dII ligation is blunt ended).

The 5′ and 3′ parts of *Mos1* were combined as in Figure 1A to create a general-purpose *Mos1* transformation vector. Plasmid pB2R was first digested with *Xho*I, the sticky ends made blunt with Klenow polymerase, and then digested a second time with *Hin*dIII to liberate the 3′ part of *Mos1* with the 0.2 kb of flanking *D. simulans* DNA. This fragment was ligated to the *Eco*RV and *Hin*dIII sites in the polylinker in pA2L to create plasmid pM789. The pM789 plasmid contains a *Mos1* element with unique *Pst*I and *Eco*RI polylinker sites inserted at the original *Sac*I site at position 789. To create the mini-*white* transformation derivative of pM789, a 4.5-kb *Pst*I fragment containing the *hsp70*:mini-*white* fusion from pMlwB (Lidholm *et al.* 1993) was inserted into the *Pst*I site of pM789 to yield the plasmid pM789[mini-*white*] (Figure 1B).

Construction of *hsp70:Mos1* and *hsp26-Sgs3:Mos1* vectors:

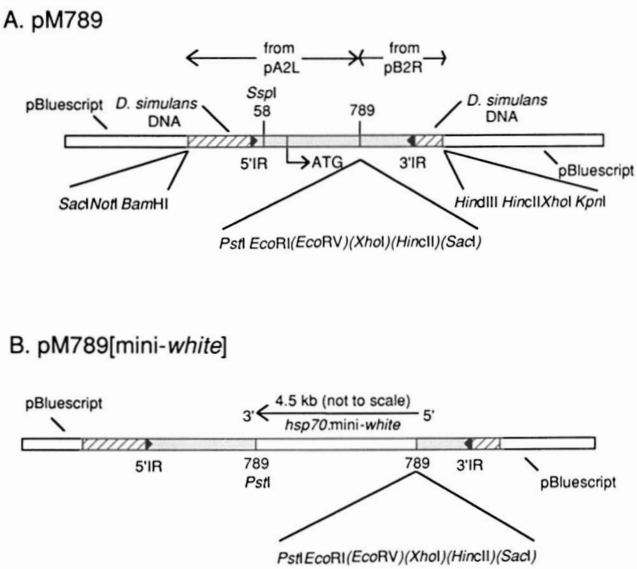

FIGURE 1.—(A) Construction of pM789 by insertion of a fragment from pB2R into pA2L (LIDHOLM *et al.* 1993). The plasmid pB2R was digested with *Xho*I, the ends made blunt with Klenow fragment, and digested again with *Hin*dIII to generate a fragment blunt at the former *Xho*I site and with a *Hin*dIII overhang at the opposite end. This fragment was ligated between the *Eco*RV and *Hin*dIII sites in pA2L. Nucleotides between the *Eco*RV and *Hin*dIII polylinker sites in pB2R were removed. The plasmid pM789 has both inverted repeats of *Mos1* and convenient *Pst*I and *Eco*RI cloning sites replacing the original *Sac*I site at *Mos1* position 789. (B) Construction of pM789[mini-*white*] by insertion of a 4.5-kb *Pst*I fragment containing mini-*white* into the *Pst*I site of pM789.

Derivatives of *Mos1* in which the coding sequence is driven by the *hsp70* promoter or the *hsp26-Sgs3* chimeric promoter were constructed from the plasmid pAd31, the structure of which is outlined in Figure 2A. The *Mos1* fragment inserted into the pAd31 polylinker was derived from a *Mos1*-containing pBluescribe M13$^+$ plasmid (Vector Cloning Systems, San Diego, CA) described in MEDHORA *et al.* (1991), which contains the *Mos1* element flanked by 3.5 kb of *D. simulans* DNA on the 5′ side and 0.2 kb of *D. simulans* DNA on the 3′ side. The orientation and numbering of *mariner* sequences are as in JACOBSON *et al.* (1986). The pBluescribe M13$^+$ plasmid was digested with *Ssp*I, which cleaves at position 58 in *Mos1*, and with *Hin*dIII to liberate a 1450-bp *Ssp*I-*Hin*dIII fragment. This fragment was ligated between the *Eco*RV and *Hin*dIII polylinker sites in pBluescript (destroying the *Eco*RV site in the process) to create plasmid pAd31. DNA fragments containing different promoters to drive *Mos1* transcription can be inserted in any of the polylinker sites upstream from *Mos1* in pAd31 (*Sac*I, *Not*I, *Bam*HI, *Sma*I, *Pst*I, and *Eco*RI). The pAd31 plasmid probably includes most, if not all, of the *Mos1* promoter elements, since the *Ssp*I site in *Mos1* is 39 bp upstream from the most 5′ element identified in the putative promoter (JACOBSON *et al.* 1986). However, the endogenous *Mos1* promoter is relatively weak (MARUYAMA and HARTL 1991b).

The *hsp70:Mos1* construct was derived by isolating a 460-bp *Bam*HI-*Eco*RI fragment containing the *hsp70* promoter and inserting this fragment between the *Bam*HI and *Eco*RI sites upstream from *Mos1* in pAd31 (Figure 2B). From the resulting plasmid (called pAd31-*hsp70:Mos1*), the *hsp70:Mos1* fusion was isolated inside a *Bam*HI-*Hin*dIII fragment, the ends made blunt with the Klenow fragment of DNA polymerase,

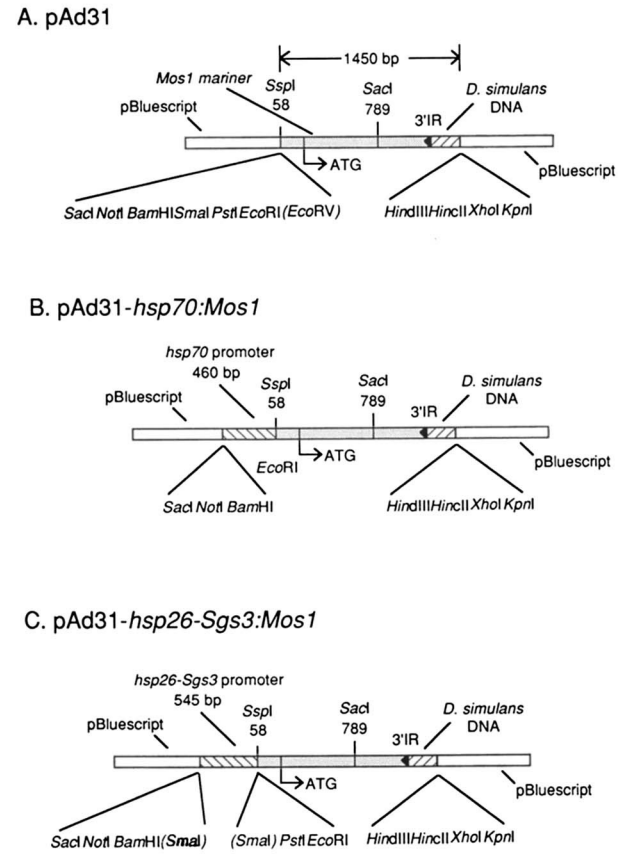

FIGURE 2.—(A) Structure of the insert in pAd31, precursor of the marooned derivatives. The restriction sites in the pBluescript polylinker are shown. In the insertion of the *Mos1* fragment, the *Ssp*I site was blunt-end ligated with the *Eco*RV fragment, destroying the *Eco*RV site in the process (indicated by the parentheses). Nucleotides between the *Eco*RV and *Hin*dIII sites in the pBluescript polylinker were removed. (B) Plasmid pAd31-*hsp70:Mos1* has a 460-bp fragment bearing the *hsp70* promoter replacing the region between the *Bam*HI and *Eco*RI polylinker sites in pAd31. (C) Plasmid pAd31-*hsp26-Sgs3:Mos1* has a 545-bp fragment bearing the *hsp26-Sgs3* chimeric promoter inserted into the *Sma*I polylinker site in pAd31.

and the fragment ligated into the *Hpa*I site of the *P*-element transformation vector Carnegie 20 (RUBIN and SPRADLING 1983). Both orientations of the insertion were obtained. We chose to focus further studies on *P*[*ry*$^+$, *hsp70:Mos1*], in which the direction of transcription of *rosy* is in the same direction as that of *Mos1*.

The *hsp26-Sgs3:Mos1* construct was derived from a 545-bp *Eco*RI-*Nhe*I fragment containing two copies of the female-germline-specific *hsp26* transcriptional regulator and the *Sgs3* basal promoter (FRANK *et al.* 1992). The ends of this fragment were made blunt with Klenow and ligated into the *Sma*I site in the polylinker in pAd31 (Figure 2C). A clone in which the *hsp26-Sgs3* promoter was driving transcription of the *Mos1* coding region was identified by DNA sequencing. From the resulting plasmid (pAd31-*hsp26-Sgs3:Mos1*), the *hsp26-Sgs3:Mos1* fusion was isolated inside a *Bam*HI-*Hin*dIII fragment, the ends made blunt, and the fragment ligated into the *Hpa*I site of Carnegie 20. The resulting *P*-element vector, designated *P*[*ry*$^+$, *hsp26-Sgs3:Mos1*] has the same direction of transcription of *rosy* and *Mos1*.

Germline transformation: Plasmid DNA was purified by CsCl-ethidium bromide equilibrium density-gradient centrifugation and used for injection of embryos. Flies of genotype *ry*^{506} were used as recipients in the transformations with Carnegie 20 derivatives; the injected DNA solution contained 450 μg/ml of the vector of interest along with 150 μg/ml of the wings-clipped helper *P* element (KARESS and RUBIN 1984). Genotype *w*^{pch} (strain P735; see below) was used for the *Mos1* transformations and *w*^{1118} for the pM789[mini-*white*] transformations. In the latter, the plasmids pM789[mini-*white*] (Figure 1B) and pBluescribe M13$^+$-*Mos1* (MEDHORA *et al.* 1991) were used in concentrations of 300 and 100 μg/ml, respectively. The baseline transformation rate of *Mos1* was estimated by injecting pBluescribe M13$^+$-*Mos1* at 500 μg/ml into embryos of genotype *w*^{pch}; *trans* complementation of the inactive element in *w*^{pch} results in excision in somatic cells and yields mosaic eye color (GARZA *et al.* 1991; MEDHORA *et al.* 1991). Plasmid pAd31-*hsp70:Mos1* was also injected into *w*^{pch} flies at 150 μg/ml to test for G0 expression of the *hsp70:Mos1* fusion, which is detected as eye-color mosaicism in the G0 adults.

Drosophila strains: All strains described are *D. melanogaster*, and all crosses were carried out at 25° on standard cornmeal-molasses medium. Descriptions of mutants and special chromosomes are found in LINDSLEY and ZIMM (1992). The strain referred to as *w*^{pch} is P735, which carries, on the *X* chromosome, a *P*-element transposon containing a chimeric *white* gene that includes part of the *w*^{pch} allele from *D. mauritiana* with an insertion of *mariner* in the promoter region (GARZA *et al.* 1991). The insertion M3–8 is an autonomous *Mos1 mariner* element inserted into chromosome *2* (GARZA *et al.* 1991). The *Mos1* element in M3–8 was originally discovered in *D. mauritiana* (BRYAN *et al.* 1987), introgressed into *D. simulans* (MEDHORA *et al.* 1988), and cloned and introduced into the germline of *D. melanogaster* by *P*-element-mediated transformation (GARZA *et al.* 1991). The *w*^{1118} allele has a deletion of part of the *white* gene.

Transpositions of *P*[*ry*$^+$, *hsp70:Mos1*]: Only two *P*[*ry*$^+$, *hsp70:Mos1*] transformants were obtained, and so mobilization by *P*-element transposase was used to create strains with different insertion sites of *P*[*ry*$^+$, *hsp70:Mos1*]. These were generated in a two-step procedure. First, an autosomal insertion of *P*[*ry*$^+$, *hsp70:Mos1*] was transposed into the *X* chromosome; then, the *X*-chromosomal insertion was transposed into an autosome (or the *Y* chromosome). The autosomal insertion used to initiate the process was the original transformant *P*[*ry*$^+$, *hsp70:Mos1*]-182, which has an insertion in chromosome *2*. From the cross *P*[*ry*$^+$, *hsp70:Mos1*]-182/*P*[*ry*$^+$, *hsp70:Mos1*]-182; *ry*^{506}/*ry*^{506} × *CyO*/*Sp*; *P*[*ry*$^+$ Δ2–3](99B), *Sb*/*TM3*, male progeny of genotype *P*[*ry*$^+$, *hsp70:Mos1*]-182/*Sp*; *P*[*ry*$^+$ Δ2–3](99B), *Sb*/*ry*^{506} were selected and crossed with *CyO*/*Sco*; *ry*^{506}/*ry*^{506} females. Progeny that are sternopleural, nonstubble, and nonrosy represent putative transpositions of *P*[*ry*$^+$, *hsp70:Mos1*]-182 to chromosomes other than chromosome *2*. Two independent transpositions to the *X* were obtained (called *P*[*ry*$^+$, *hsp70:Mos1*]-X1 and *P*[*ry*$^+$, *hsp70:Mos1*]-X2).

To obtain additional insertions sites of *P*[*ry*$^+$, *hsp70:Mos1*], males of genotype *P*[*ry*$^+$, *hsp70:Mos1*]-X1/*Y*; *P*[*ry*$^+$ Δ2–3](99B), *Sb*/*ry*^{506} were crossed with females of genotype *y*; *ry*^{506}. Males that are nonstubble and nonrosy represent putative transpositions of *P*[*ry*$^+$, *hsp70:Mos1*] to a chromosome other than the *X*. From ~250 crosses, independent transpositions of *P*[*ry*$^+$, *hsp70:Mos1*] to 121 locations in autosomes and one in the *Y* chromosome were isolated.

RESULTS

Baseline efficiency of transformation: The *w*^{pch} allele results in a peach-colored eye; the allele contains an

TABLE 1

Efficiency of transformation by the *Mos1* element

	No. fertile G0	No. transformants	Percent transformants
Experiment 1	37	5	14
Experiment 2	14	3	21
Total	51	8	16

inactive *mariner* element (called *peach*) in the region of the *white* promoter and provides a convenient assay for *trans* complementation by autonomous *mariner* elements elsewhere in the genome (CAPY *et al.* 1990; JACOBSON *et al.* 1986). In the presence of an active *mariner* element, the *peach* element is excised in a proportion of the somatic cells during development. Excision of *peach* in pigment cells results in restoration of red eye pigmentation. Hence, w^{bch} flies containing a *trans*-complementing *mariner* element have eye-color mosaicism appearing as red flecks or patches in a background of peach. Germline excision of *peach* usually results in a wild-type eye-color phenotype. It should be emphasized that the *trans* complementation of *peach* excision has so far been examined only with respect to autonomous elements in the mauritiana subfamily closely related to *peach*, from which *Mos1* differs by 11 bp including four amino acid replacements (MEDHORA *et al.* 1991). It is unclear how much sequence divergence between an autonomous element and a nonautonomous element is still compatible with *trans* complementation.

The *Mos1* element is an autonomous *mariner* element isolated from *D. mauritiana* (MEDHORA *et al.* 1991). When injected into embryos of *D. melanogaster*, *Mos1* can integrate into the genome. However, in previous control experiments carried out in a rather small scale, the efficiency of *Mos1* transformation varied from 31% (five transformants among 16 fertile G0 flies; GARZA *et al.* 1991) to 4% (one transformant among 23 fertile G0 flies; LIDHOLM *et al.* 1993). To establish a baseline for efficiency of *Mos1* transformation in *D. melanogaster*, *Mos1* was injected into w^{bch} embryos, and the eye color of the adult survivors was examined. Germline integration of *Mos1* results in mosaic eyes in G1 progeny. Germline excision of the w^{bch} element resulting from the injected *Mos1* DNA yields wild-type eyes in G1 progeny.

The results of two experiments with injections at a concentration of 500 μg/ml are shown in Table 1. Previous experiments had used 150 μg/ml. Averaging 16% (with a binomial standard error of 5%), the levels of *Mos1* transformation are comparable with those typically obtained with the *P* element (RUBIN and SPRADLING 1982). Of particular interest is that the probability of transformation was highly correlated with the appearance of red-eyed germline revertants in the same cultures. Among the 51 w^{bch} fertile G0 flies crossed with uninjected w^{bch} flies, 17 (33%) cultures produced at least one red-eyed progeny. All eight germline transformants were obtained from the 17 cultures that also showed germline excision ($P < 0.001$). This finding suggests that only a proportion of the injected embryos are competent either to take up the injected DNA or to integrate it to yield transformants.

Transformation with M789[mini-*white*]: In the absence of exogenous DNA, *Mos1* transforms the *D. melanogaster* w^{bch} strain with an efficiency of about 16% (Table 1). Yet the transformation efficiency was reduced ~20-fold when *Mos1* was used to support germline transformation with the 13.2-kb construct MlwB having 11.9 kb of exogenous DNA inserted at the *Sac*I site at position 789 (LIDHOLM *et al.* 1993). One hypothesis to explain the reduced transformation efficiency is that the 13.2-kb size of the MlwB transposon is excessive. The M789[mini-*white*] transposon was therefore constructed (Figure 1B), which, with an overall size of 5.8 kb, is less than half the size of the MlwB transposon. The *Mos1* element was used as the helper in transformation to enable comparison with the transformation of MlwB. Among 320 fertile G0 flies, two red-eyed transformants were obtained. This 0.6% efficiency is essentially identical to that observed with MlwB. One M789[mini-*white*] transformant (designated M256) mapped to chromosome *3* and was associated with a recessive lethality. The second transformant (M325) mapped to the *X* chromosome in salivary chromosome region 3B, where it was associated with recessive female sterility. These results show that the transformation efficiency is not detectably different whether the length of exogenous DNA inserted into the *Sac*I site is 4.5 or 11.9 kb.

To facilitate further genetic analysis of these and other transformants, we produced *mariner* derivatives in which transcription of the transposase-coding region is driven by either of two efficient promoters and in which the 5′ inverted repeat is missing to prevent self-mobilization by the transposase. These constructs are described in the following.

***P*[*ry*$^+$, *hsp70:Mos1*]:** A stable genomic source of *Mos1* transposase was produced by fusing an *hsp70* promoter to the 5′ end of the *mariner* coding region replacing the 5′ inverted repeat up to position 58 (Figure 2B). The *hsp70:Mos1* fusion has tandem promoters because it still retains the putative promoter elements of *Mos1*. The *Mos1* promoter is, however, relatively weak (MARUYAMA and HARTL 1991b).

To test the effectiveness of the *hsp70:Mos1* construct, the plasmid pAd31-*hsp70:Mos1* was injected into embryos of genotype w^{bch} at 150 μg/ml. Some of the surviving G0 adults showed eye-color mosaicism, an effect resembling the maternal effect shown in the non-*Mos1* progeny of w^{bch} females heterozygous for *Mos1* (BRYAN and HARTL 1988). This phenomenon is analogous to the finding of nonrosy phenotypes among G0 adults injected with Carnegie 20 and results from transient

expression of the injected DNA. In this case, either the *Mos1* transcript or the putative transposase remains active enough to cause *peach* excision during differentiation of the eye imaginal disc in w^{pch} embryos. Among the progeny of 25 fertile G0 adults, three (12%) yielded one or more progeny with red eyes resulting from excision of the *peach* element in the germline and restoration of wild-type gene function. These results show that the *hsp70:Mos1* element can function as a source of transposase at high enough levels to excise the *peach* element both in the soma and in the germline.

A stable genomic source of *Mos1* transposase was obtained from integration of the *P*[ry^+, *hsp70:Mos1*] transposon after injection into ry^{506} together with the wings-clipped helper element. From 398 G0 adults, two germline transformants were obtained. One of the insertions, *P*[ry^+, *hsp70:Mos1*]-182 was mapped to polytene region 33C6 on chromosome *2*. When crossed with w^{pch}, *P*[ry^+, *hsp70:Mos1*]-182 yields an extreme level of somatic eye-color mosaicism even in the absence of heat shock to induce the *hsp70* promoter. To examine possible position effects on *P*[ry^+, *hsp70:Mos1*] expression, the construct was transposed to multiple locations in the genome. Using the mating scheme outlined in MATERIALS AND METHODS, *P*[ry^+, *hsp70:Mos1*]-182 was first transposed to the *X* chromosome using *P*[ry^+ 2–3] (99B) and then transposed to 122 different locations in chromosomes other than the *X*. With one exception, all 122 insertions yielded very high levels of somatic mosaicism of w^{pch} even in the absence of heat shock. The exceptional insertion (*P*[ry^+, *hsp70:Mos1*]-55) showed variegation of the ry^+ marker and weak w^{pch} mosaicism in the male progeny and proved to be an insertion into the *Y* chromosome.

Although capable of mobilization by the *P*-element transposase, all of the *P*[ry^+, *hsp70:Mos1*] insertions lack the 5′ inverted repeat of *Mos1* and so are unable to be excised or transposed by the *Mos1* transposase. We refer to the *hsp70:Mos1* components of these elements as "marooned" owing to their inability to transpose independently of the *P*[ry^+, *hsp70:Mos1*] transposon containing them.

***P*[ry^+, *hsp26-Sgs3:Mos1*]:** A strong germline promoter of *Mos1* transcription was also deemed desirable for possible use in transformation. For this purpose we constructed a *P*[ry^+, *hsp26-Sgs3:Mos1*] transposon with the chimeric *Sgs3* promoter-*hsp26* enhancer fused to *Mos1* replacing the 5′ inverted repeat up to position 58 (Figure 2C). The *Sgs3* promoter is a basal promoter and the *hsp26* enhancer is apparently specific for nurse cells and the oocyte (FRANK *et al.* 1992). The *hsp26-Sgs3:Mos1* fusion has tandem promoters, however, because the promoter elements of *Mos1* remain intact. Lacking the 5′ inverted repeat of *Mos1*, the *hsp26-Sgs3:Mos1* fusion is marooned with respect to *mariner* transposase.

Transformation of ry^{506} with Carnegie 20 containing *P*[ry^+, *hsp26-Sgs3:Mos1*] yielded 30 independent transformants. Ten of these were examined with parents of both sexes in the w^{pch} somatic excision assay. All of the tested insertions exhibited strong maternal effects on eye-color mosaicism of w^{pch} in most of the progeny (typically 80–100%), which is observed as large blotches or streaks of pigmentation. The maternal effect presumably arises from the female germline activity of the *hsp26-Sgs3* promoter. On the other hand, with one exception, the progeny of males showed little eye-color mosaicism of w^{pch} and was manifested as individual pigmented ommatidia in a salt and pepper pattern, typically <10 pigmented ommatidia per eye. The exceptional line (*P*[ry^+, *hsp26-Sgs3:Mos1*]-38) showed strong somatic mosaicism in the progeny of males, comparable to that observed in the *P*[ry^+, *hsp70:Mos1*] insertions. The somatic expression in this case presumably arises from the trapping of an eye-specific enhancer by the *hsp26-Sgs3* promoter or the *Mos1* promoter. The generally weak somatic expression of the *hsp26-Sgs3* promoter was expected from the reported female-germline-specific activity of the *hsp26-Sgs3* promoter and the generally weak activity of the *Mos1* promoter.

Comparison of *P*[ry^+, *hsp70:Mos1*] and *P*[ry^+, *hsp26-Sgs3:Mos1*]: Quantitative comparisons of the efficacy of the *hsp70:Mos1* and *hsp26-Sgs3:Mos1* fusions were obtained from rates of germline excision of *peach* from the w^{pch} allele. Data from tested lines are summarized in Table 2, in which the symbol [*hsp26-Sgs3*]-i and [*hsp70*]-i denote the insertions *P*[ry^+, *hsp26-Sgs3:Mos1*]-i and *P*[ry^+, *hsp70:Mos1*]-i, respectively. *P*[ry^+, *hsp70:Mos1*]-55 is an insertion into the *Y* chromosome, and M3–8 is a highly expressed insertion of *Mos1* in chromosome *2* (GARZA *et al.* 1991). When the tested parent was a male, the genotype with respect to *X*-chromosomal markers was *y* w^{pch}/Y, and so the tabulated number of F_1 progeny includes only females. When the tested parent was a female, the *X*-chromosomal genotype was *y* w^{pch}/*y* w^{pch} and, in this case, the tabulated number of F_1 progeny includes both sexes. Note that the number of copies of the *peach* target is the same in the tabulated progeny in both crosses.

The data in Table 2 establish two unexpected results: there is no detectable dosage effect of the *hsp26-Sgs3:Mos1* fusion on the rate of germline excision—homozygotes exhibit about the same level as heterozygotes and the rate of germline excision in the *hsp26-Sgs3:Mos1* construct is as high in males as it is in females.

These comparisons of heterozygotes and homozygotes are illustrated in Figure 3. In the case of *hsp26-Sgs3:Mos1* constructs, four of five tested showed an increase in the excision rate in homozygous flies but in only one (insertion number 97) was the difference statistically significant in a chi-square test ($P < 0.05$). The difference in reversion rate between homozygotes and heterozygotes is quite small and not statistically significant in the group as a whole. The data from *hsp26-*

TABLE 2

Germline excision of *peach* element

Line	Parental genotype	Sex of parent	Total F_1 progeny	Red-eyed flies	F_1 reversion (%)
[*hsp26-Sgs3*]-28	*28/28*	Male	507	71	14.0
	28/TM3	Male	446	64	14.3
	28/28	Female	983	170	17.3
	28/TM3	Female	ND	ND	ND
[*hsp26-Sgs3*]-34	*43/43*	Male	413	70	16.9
	43/CyO	Male	462	97	21.0
	43/43	Female	145	29	20.0
	43/CyO	Female	156	36	23.1
[*hsp26-Sgs3*]-67	*67/67*	Male	147	30	20.4
	67/CyO	Male	386	84	21.8
	67/67	Female	244	61	25.0
	67/CyO	Female	ND	ND	ND
[*hsp26-Sgs3*]-97	*97/97*	Male	395	91	23.0
	97/TM3	Male	398	50	12.6
	97/97	Female	1006	198	19.7
	97/TM3	Female	591	113	19.1
[*hsp26-Sgs3*]-107	*107/107*	Male	ND	ND	ND
	107/CyO	Male	441	78	17.7
	107/107	Female	ND	ND	ND
	107/CyO	Female	615	79	12.8
[*hsp26-Sgs3*]-110	*110/110*	Male	395	63	15.9
	110/CyO	Male	413	63	15.3
	110/110	Female	56	14	25.0
	110/CyO	Female	ND	ND	ND
[*hsp70*]-182	*182/182*	Male	324	43	13.3
	182/CyO	Male	182	31	17.0
	182/182	Female	609	78	12.8
	182/CyO	Female	392	55	14.0
[*hsp70*]-56	*56/56*	Male	345	36	10.4
	56/TM3	Male	355	46	13.0
	56/56	Female	598	42	7.0
	56/TM3	Female	728	95	13.0
M3-8 (*Mos1*)	*M3-8/CyO*	Male	275	38	13.8
	M3-8/CyO	Female	487	42	8.6
[*hsp70*]-55 (*Y*)	*55/Y*	Male	442	47	10.6

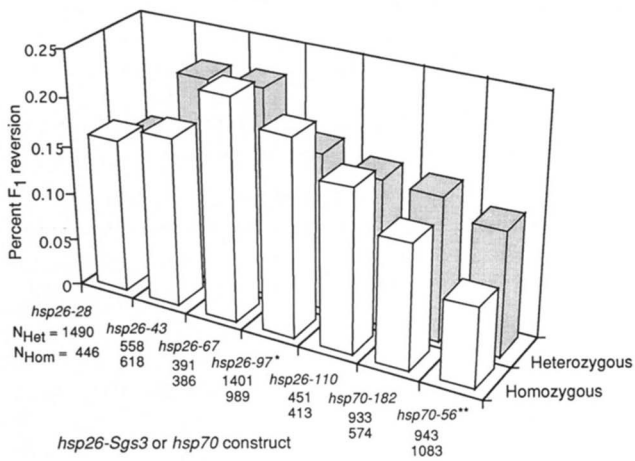

FIGURE 3.—Comparison of rate of germline excision of the *peach* element in heterozygotes and homozygotes carrying various *hsp26-Sgs3:Mos1* and *hsp70:Mos1* fusions.

Sgs3:Mos1 therefore suggest a saturation effect in which, at a *peach* excision rate of somewhat <20%, doubling the number of copies of *Mos1* causes no detectable increase in excision rate. The *hsp70:Mos1* constructs were quite strange: both showed a decreased excision rate in homozygotes (Figure 3, in one case statistically highly significant), and the pooled data are also significant in suggesting a somewhat lower excision rate in homozygotes.

Comparisons of *peach* excision rates in males and females are illustrated in Figure 4. There is no indication of a different rate of excision in *hsp26-Sgs3:Mos1* males *vs.* females. None of the individual comparisons is statistically significant, and while four out of five show a slight increase in females, $P = 0.16$ in a one-tailed nonparametric test. The pooled data (Table 3) support this conclusion.

On the other hand, while the *hsp26-Sgs3:Mos1* fusion does not show female-germline specificity, it is much

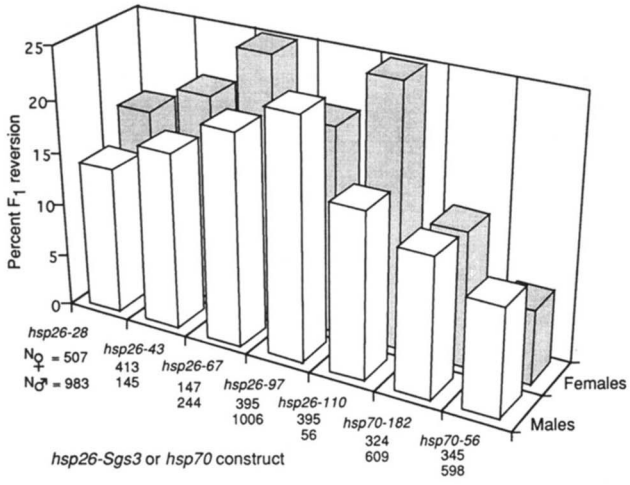

FIGURE 4.—Comparison of rate of germline excision of the *peach* element in males and females carrying various *hsp26-Sgs3:Mos1* and *hsp70:Mos1* fusions.

more active in the germline than either *Mos1* itself or *hsp70:Mos1*. This conclusion is shown by the three comparisons at the bottom of Table 3. The excision rate with the *hsp26-Sgs3:Mos1* promoter is almost twofold greater than with *Mos1* in the strong-expression strain M3–8. Similarly, when compared with *hsp70:Mos1*, the *hsp26-Sgs3:Mos1* promoter exhibits a *peach* excision rate ~25% greater in heterozygotes and ~75% greater in homozygotes (Table 3). All of these effects are highly statistically significant.

Grand-maternal effects on *peach* excision: As noted, females expressing the *Mos1* transposase have progeny in which maternal effects on *peach* excision result in mosaic eye color of w^{pch}. With such strong maternal effects in the soma, it is reasonable to expect that the germline of the progeny might also be affected. Operationally, the phenomenon would be expressed as a grand-maternal effect in which the phenotype of the F_2 progeny depends on the genotype of their grandmother. In other words, maternal effects on the progeny germline could be detected experimentally as an increased rate of w^{pch} reversion in the F_2 generation.

To determine the extent to which a grand-maternal effect on *peach* excision accounts for the apparent activity of *hsp:Mos1*, we carried out the experiment outlined in Figure 5. Females heterozygous for either *hsp70:Mos1* or *hsp26-Sgs3:Mos1* were mated with balancer (*Bal1/Bal2*) males and their progeny (w^{pch}/w^{1118} females or w^{pch}/Y males) tested to determine the rate of w^{pch} reversion. The histograms in Figure 5 depict the reversion rates for *P*[*ry*$^+$, *hsp70:Mos1*]-56 and *P*[*ry*$^+$, *hsp70:Mos1*]-182 (open bars) and *P*[*ry*$^+$, *hsp26-Sgs3:Mos1*]-38, *P*[*ry*$^+$, *hsp26-Sgs3:Mos1*]-67, and *P*[*ry*$^+$, *hsp26-Sgs3:Mos1*]-97 (shaded bars). The numbers in parentheses by each class of progeny are the average reversion rates for *hsp70:Mos1* (top) and *hsp26-Sgs3:Mos1* (bottom). The grand-maternal effect is evident in the F_1 *Bal1/Bal2* progeny, which yield rates of w^{pch} reversion ranging from 2 to 6%; in comparable experiments with no *hsp:Mos1* in the ancestry, the rate of w^{pch} reversion is 0 (data not shown).

Although the grand-maternal effect on w^{pch} excision is significant, it accounts for only about one-fourth of the total excision rate observed in F_1 *hsp:Mos1* males (28%, averaged over both *hsp70:Mos1* and *hsp26-*

TABLE 3

Comparisons of germline excision rates

Comparison		No. nonrevertant progeny	No. revertant progeny	Percent revertant	χ^2
Mos1 (M3-8)	Heterozygous male	237	28	10.6	
	Heterozygous female	445	42	8.6	0.77
hsp26-Sgs3:Mos1	Male	3642	761	17.3	
	Female	3096	700	18.4	1.86
hsp26-Sgs3:Mos1	Heterozygous	3244	664	17.0	
	Homozygous	3494	797	18.6	3.50
hsp70:Mos1	Male	1050	156	12.9	
	Female	2057	270	11.6	1.33
hsp70:Mos1	Heterozygous	1430	227	13.7	
	Homozygous	1677	199	10.6	7.93**
hsp70:Mos1 vs. Mos1	*hsp70:Mos1* heterozygous	1430	227	13.7	
	Mos1 heterozygous	682	70	9.3	9.23**
hsp26-Sgs3:Mos1 vs. Mos1	*hsp26-Sgs3:Mos1*	6738	1461	17.8	
	Mos1 heterozygous	682	70	9.3	35.19***
Heterozygous	*hsp26-Sgs3:Mos1*	3244	664	17.0	
	hsp70:Mos1	1430	227	13.7	9.37**
Homozygous	*hsp26-Sgs3:Mos1*	3494	797	18.6	
	hsp70:Mos1	1677	199	10.6	61.17***

** $P < 0.01$; *** $P < 0.001$.

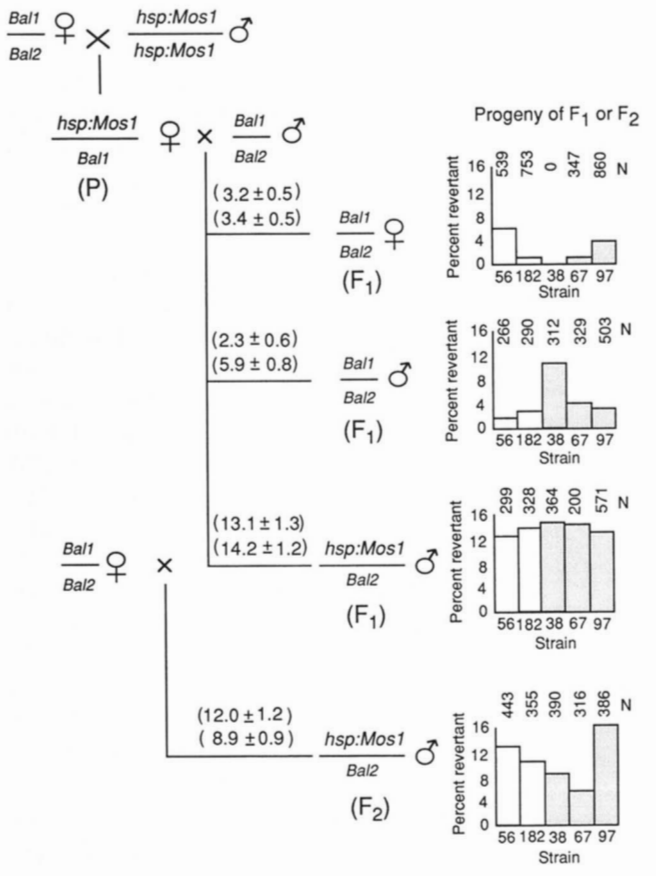

FIGURE 5.—Demonstration of grand-maternal effect of *hsp:Mos1*. The top three histograms show the w^{bch} reversion rates among the progeny of F_1 flies whose mother was heterozygous for *hsp:Mos1*. The numbers labeling each bar of the histograms represent the transformants *P*[ry^+, *hsp70:Mos1*]-56 and -182 (open bars) and *P*[ry^+, *hsp26-Sgs3:Mos1*]-38, -67, and -97 (shaded bars). Transformants -182 and -67 have the *hsp:Mos1* fusion inserted in chromosome *2*; lines -56, -38, and -97 have it inserted in chromosome *3*. *N*, number of w^+ and w^{bch} progeny observed in each cross. The numbers in parentheses at the left are the average reversion rates for *hsp70:Mos1* (top) and *hsp26-Sgs3:Mos1* (bottom) for each type of F_1 progeny. The grand-maternal effect is indicated by the nonzero *peach* reversion rates in the progeny of balancer (*Bal1/Bal2*) F_1 progeny. Of the total *peach* reversion rate observed in F_1 *hsp:Mos1* males, about one-fourth is attributable to the grand-maternal effect. The F_2 males at the bottom show the direct genotypic effects of *hsp:Mos1* on *peach* excision without the complication of the grand-maternal effect. For the second chromosome, the balancers were *CyO (Bal1)* and *Sco (Bal2)*; for the third chromosome, *TM3, Sb (Bal1)* and *D (Bal2)*. Only the *Bal1* chromosomes were used for balancing in females.

Sgs3:Mos1). The direct phenotypic effects of the *hsp:Mos1* fusions, uncomplicated by the grand-maternal effects, are seen in the reversion rates in the progeny of the F_2 *hsp:Mos1/Bal2* sons obtained from F_1 *hsp:Mos1/Bal2* fathers. In this cross, the *hsp:Mos1* chromosome was present in the paternal grandfather of the F_3 progeny, and so the grand-maternal effect is eliminated. As expected, the reversion rate in F_2 *hsp:Mos1/Bal2* males is somewhat lower than that observed in the F_1 males owing to the additional grand-maternal effect present in the F_1 males.

Effects of *hsp:Mos1* fusions on MlwB and mini-*white* transformants: The *hsp:Mos1* fusions were created in part to ascertain the somatic stability of the MlwB (LIDHOLM *et al.* 1993) and M789[mini-*white*] (this paper) transformants in the presence of a transposase source driven by a strong promoter. Accordingly, the MlwB transformants M108 and M159 and the M789[mini-*white*] transformants M256 and M325 were all examined for somatic eye-color mosaicism in the presence of either *hsp70:Mos1* or *hsp26-Sgs3:Mos1*. No evidence of eye-color mosaicism was found in any of the transformants apart from an occasional fly with a single patch of white tissue. None of the transformants displayed somatic instability at anywhere near the level observed in w^{bch} flies in the presence of these transposase sources. To rule out the possibility of position effects on the transposase source, the *P*[ry^+, *hsp70:Mos1*]-182 transposon was mobilized by *P*-element transposase, and 121 independent sites of insertion in the autosomes were tested for their ability to induce somatic mosaicism in either M108 and M159. None of the new insertion sites gave evidence of increased somatic instability of the MlwB insertions.

DISCUSSION

Stable sources of *Mos1* transposase: Both the *hsp70:Mos1* and *hsp26-Sgs3:Mos1* fusions provide genetically stable sources of *Mos1* transposase in *D. melanogaster*. The *hsp70:Mos1* fusion is much more active in the soma than is the *hsp26-Sgs3:Mos1* fusion, but the latter exhibits a very strong maternal effect on *peach* excision. We have also shown that injection of the pAd31-*hsp70:Mos1* plasmid into embryos results in somatic mosaicism of w^{bch} in G0 adults as well as germline excision of the *peach* element visible in G1 progeny. Such effects are expected of transient expression of the injected DNA. Similar experiments have not been carried out with the pAd31-*hsp26-Sgs3:Mos1* plasmid because it is active only in germline cells of adults. Germline effects of this construct are expected in progeny in view of the greater rate of germline excision resulting from *hsp26-Sgs3:Mos1* as compared with *hsp70:Mos1* when these fusions are integrated into the genome. It is our hope that these plasmids will prove useful to researchers attempting *mariner*-mediated germline transformation in other species.

Lack of dosage effect in heterozygotes: The lack of any significant difference in the rate of *peach* germline excision in *hsp26-Sgs3:Mos1* homozyotes and heterozygotes was unexpected but explicable on the basis of a saturation effect in which, at high enough levels of transposase, factors other than transposase availability are rate limiting to excision. The same explanation may account for the similar excision rate observed in homozygous w^{bch} females and hemizygous w^{bch} males, in spite

of the differing number of copies of the w^{pch} target. More puzzling is the somewhat greater rate of germline excision observed in *hsp70:Mos1* heterozygotes than in homozygotes. One possibility is that the finding is a peculiar property of the particular *hsp70:Mos1* strains chosen for examination. Indeed, all of the statistical significance comes from the one insertion *P*[*ry*$^+$, *hsp70:Mos1*]-56. Perhaps this particular insertion operates under a kind of transvection effect in which the total rate of transcription from a single copy is somewhat greater than that from two paired copies. With respect to the other insertion examined (*P*[*ry*$^+$, *hsp70:Mos1*]-182), there is also a slight excess of *peach* excision in heterozygotes, but it is not statistically significant.

Male germline activity of *hsp26-Sgs3:Mos1* fusion: Most surprising to us was the high rate of germline excision of *peach* in males of all the *hsp26-Sgs3:Mos1* strains examined, particularly when contrasted with the weak level of somatic mosaicism observed in the males. Studies of β-galactosidase expression have indicated that the *hsp26-Sgs3* promoter in this fusion shows a high level of specificity for expression only in nondividing cells of the female germline, particularly nurse cells and oocytes (FRANK *et al.* 1992). Our finding of a marked maternal effect on *peach* excision is consistent with a high level of expression in these cell types. The ability to detect the expression of the *hsp26-Sgs3* construct in the male germline might simply reflect the high level of sensitivity of the *peach* excision assay and indicate that the *hsp26* transcriptional regulator is generally germline specific rather than exclusively female-germline specific. On the other hand, the *hsp26-Sgs3:Mos1* constructs actually have tandem promoters (*hsp26-Sgs3* and *Mos1*), and the high rate of *peach* excision in the male germline may result from an interaction between the *hsp26-Sgs3* and *Mos1* promoters rather than from a direct effect of the *hsp26-Sgs3* promoter itself.

Grand-maternal effects on excision: The *mariner* transposase (or its mRNA) is maternally transmitted to the egg and results in extensive eye-color mosaicism in w^{pch} progeny. Our studies with *hsp70:Mos1* and *hsp26-Sgs3:Mos1* have also revealed a grand-maternal effect mediated by transposase in the germline of the F_1 progeny that is detected as an increased rate of *peach* excision in the F_2 grandprogeny. The grand-maternal effect alone results in an average excision rate of ~4% in the F_2 progeny, compared with an average excision rate of 11% caused by a direct genotypic effect of *hsp:Mos1*. The grand-maternal effect is not larger in *hsp70-Sgs3:Mos1* than it is in *hsp70:Mos1*.

Implications for *mariner* transformation: The value of 16% for autonomous *Mos1* transformation efficiency when injected at 500 μg/ml is comparable to that observed with *P*-element transformations. Such a high frequency would make it feasible to attempt to introduce *Mos1* into the genome of any target species of interest merely by injecting embryos at an appropriate stage and screening G1 progeny for the presence of *Mos1* by the polymerase chain reaction. Indeed, using this method we have been able to introduce *Mos1* into the genome of *D. virilis* (A. R. LOHE and D. L. HARTL, unpublished data), a result that hopefully presages the ability to transform *D. virilis* and other species with exogenous DNA.

Mos1-mediated transformation of vectors containing exogenous DNA results in an ~20-fold decrease in transformation efficiency as compared with *Mos1* itself. This phenomenon was reported previously with the 13.2-kb construct MlwB (LIDHOLM *et al.* 1993) and is confirmed here with the 5.8-kb construct M789[mini-*white*]. Both of these vectors have the exogenous DNA inserted in *Mos1* at the *Sad* site at position 789. The transformation efficiency is not detectably increased when the size of the exogenous DNA fragment is substantially reduced. Moreover, both types of insertions are somatically quite stable even when the source of transposase is provided by *hsp70:Mos1* or *hsp26-Sgs3:Mos1*. Could it be the case that the overall size of a *mariner* construct must be close to 1.3 kb for efficient transposition? Or do the reduced transformation efficiency and increased somatic stability both result from perturbation of the nucleotide sequence around the *Sad* site? Additional vectors with insertions at other positions in *Mos1* are currently being evaluated to test these possibilities.

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