Hermes, a Functional Non-Drosophilid Insect Gene Vector From *Musca domestica*

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ABSTRACT

Hermes is a short inverted repeat-type transposable element from the house fly, *Musca domestica*. Using an extra-chromosomal transpositional recombination assay, we show that *Hermes* elements can accurately transpose in *M. domestica* embryos. To test the ability of *Hermes* to function in species distantly related to *M. domestica* we used a nonautonomous *Hermes* element containing the *Drosophila melanogaster white* (w^+) gene and created *D. melanogaster* germline transformants. Transgenic G_1 insects were recovered from 34.6% of the fertile G_0 adults developing from microinjected w^- embryos. This transformation rate is comparable with that observed using *P* or *hobo* vectors in *D. melanogaster*, however, many instances of multiple-element insertions and large clusters were observed. Genetic mapping, Southern blotting, polytene chromosome *in situ* hybridization and DNA sequence analyses confirmed that *Hermes* elements were chromosomally integrated in transgenic insects. Our data demonstrate that *Hermes* elements transpose at high rates in *D. melanogaster* and may be an effective gene vector and gene-tagging agent in this species and distantly related species of medical and agricultural importance.

DEVELOPING genetic transformation technology for insects of medical and agricultural importance is a central unsolved problem in entomology (WALKER 1989; CRAMPTON *et al.* 1990; EGGLESTON 1991; HANDLER and O'BROCHTA 1991; O'BROCHTA and HANDLER 1993; WARREN and CRAMPTON 1994). This technology would promote insect molecular genetics research, the development of new insect pest management strategies and reduce our dependance on chemically based insecticides. In addition, genetic transformation technology would increase the scope and efficiency of current biological control strategies by permitting rational genotype modification of beneficial insects and arthropods (HOY 1993). Despite the interest in this technology and the considerable efforts expended toward developing it only limited progress has been made.

The popular *D. melanogaster* gene vector, *P*, has been tested as a gene vector in a number of systems, including non-drosophilid insects and mammalian cells. Although transgenic non-drosophilid insects and mammalian cells were occasionally recovered using *P*-element-containing vectors, none resulted from transpositional recombination mediated by *P* elements or *P* transposase (CLOUGH *et al.* 1985; KHILLAN *et al* 1985; MILLER *et al.* 1987; MCCRANE *et al.* 1988; MORRIS *et al.* 1989). The mobility of *P* elements isolated from *D. melanogaster* is confined largely to those species of *Drosophila* most related to *melanogaster* and *P* will not be a useful non-drosophilid insect gene vector without modification (O'BROCHTA and HANDLER 1988). Other transposable elements isolated from *Drosophila* have shown more promise as potential gene vectors in non-drosophilid insects.

Members of the *mariner/Tc1* family of elements appear capable of transposition when introduced into "non-host" species. Extensive analysis of the distribution of these elements provides strong evidence for the recent introduction and spread of these elements in diverse taxa (MARUYAMA and HARTL 1991; ROBERTSON 1993; ROBERTSON and MACLEOD 1993). Based on the number of examples of possible interspecific movement of *mariner/Tc1* elements between diverse insect species it appears that these elements are capable of transposition when introduced into heterologous hosts. Recently, we reported data demonstrating that the *mariner* element from *D. mauritiana* is capable of transposase-mediated excision when introduced into the embryos of the widely diverged species, *Lucilia cuprina* (family, Calliphoridae) (COATES *et al.* 1996a). The *mariner* excision footprints recovered from *L. cuprina* were identical to footprints recovered after excision from *D. melanogaster* and *D. mauritiana*. These excision-reaction products reflect "normal" *mariner* activity in *L. cuprina* suggesting that this element may be useful as a gene vector in this species. *Minos*, a *mariner/Tc1* type element from *D. hydei* (FRANZ and SAVAKIS 1991; FRANZ *et al.* 1994), can transpose when introduced into *D. melanogaster* and more recent experiments have resulted in *Minos* inte-

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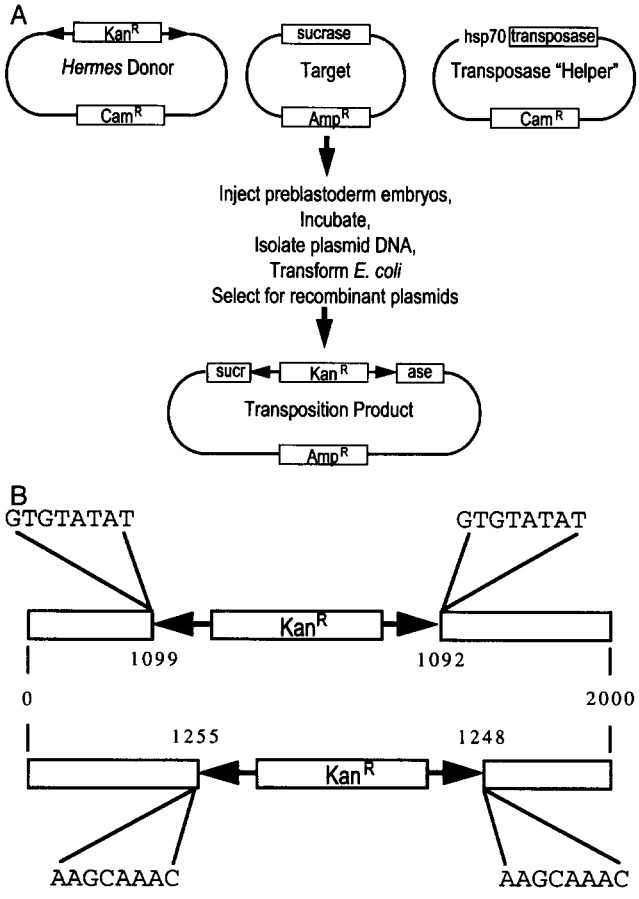

FIGURE 1.—*In vivo* extrachromosomal *Hermes* transposition assay. (A) *Hermes*-donor (p*Hermes*Kan), *Hermes*-helper (pBCHSHH1.9) and target (pUCsacRB) plasmids were co-injected into preblastoderm *M. domestica* embryos, subsequently recovered and transformed into *E. coli*. Colonies carrying recombinant plasmids in which the *Hermes* element had transposed from the donor into the target plasmid and insertionally inactivated the levansucrase gene were selected on kanamycin, ampicillin and sucrose containing media. (B) The structure of two independent *Hermes* transpositions into the *sacRB* target recovered from *M. domestica* embryos. Only sequences precisely delimited by the terminal inverted repeats of *Hermes* transposed and integrations generated 8-bp target site duplications, the sequences of which are shown. Note that *Hermes* inserted in the opposite orientation relative to the *sacRB* sequence in the lower of the two cases. Unlabeled open boxes represent the levansucrase coding region and numbers correspond to the sequence given in GenBank accession X02730. Arrowheads represent the left and right ends of *Hermes* and labeled open boxes depict relevant coding regions. Diagrams are not to scale.

gration into the genome of the distantly related species, *Ceratitis capitata* (family, Tephritidae) (LOUKERIS *et al.* 1995). Therefore, the *mariner/Tc1* family of elements may be a source of non-drosophilid insect gene vectors.

The ability to transpose in species quite diverged from their hosts appears to be a common feature of members of the *hobo, Ac, Tam3* (*hAT*) family of elements. The *Ac* element from *Zea mays* has a very broad host range and has led to its use as a gene tagging agent in a growing number of plant species including Petunia (CHUCK *et al.* 1993), tomato (PETERSON and YODER 1993), and Arabidopsis (AARTS *et al.* 1993), while the *Tam3* element from *Antirrhinum majus* has also been shown to function in several plant species (MARTIN *et al.* 1989). Likewise, the *hobo* element from *D. melanogaster* has the ability to transpose when introduced into diverged insect species such as the housefly *Musca domestica* (family, Muscidae), the Queensland fruitfly, *Bactrocera tryoni* (family, Tephritidae) and the lepidoptera *Helicoverpa armigera* (family, Noctuidae) (O'BROCHTA *et al.* 1994; S. WHYARD, H. A. MENDE, A. C. PINKERTON, C. J. COATES, D. A. O'BROCHTA and P. W. ATKINSON, unpublished data). Transposons of the *hAT* family are widespread (JOHNS 1990; MACRAE *et al.* 1990; CALVI *et al.* 1991; FELDMAR and KUNZE 1991) and representatives are found in non-drosophilid insects (ATKINSON *et al.* 1993; WARREN *et al.* 1994, 1995; COATES *et al.* 1996b). Based on these observations *hAT* elements have great potential to serve as non-drosophilid insect gene vectors.

The *Hermes* transposable element is a member of the *hAT* family of transposable elements and was isolated from the housefly, *Musca domestica* (ATKINSON *et al.* 1993; WARREN *et al.* 1994). Full-length *Hermes* elements are 2749 bp in length and contain a single open reading frame capable of encoding a protein 613 amino acids in length. This protein is 55% identical and 71% similar to the transposase encoded by the open reading frame of the *hobo* transposable element of *D. melanogaster*. *Hermes* elements contain 17 bp imperfect terminal inverted repeats that strongly resemble the terminal repeats of *hobo* and other *hAT* elements (WARREN *et al.* 1994). Copy number, length and insertion site polymorphisms have also been observed for *Hermes* elements in a number of different *M. domestica* strains, indicating that *Hermes* elements are active or were recently active (ATKINSON *et al.* 1993; WARREN *et al.* 1994). Here we report data demonstrating directly that *Hermes* elements can transpose in *M. domestica* and are capable of serving as an efficient germline transformation vector in *D. melanogaster*, a species that diverged from *Musca* ~100 mya (HENNIG 1981). *Hermes* is the first functional non-drosophilid insect transposable element shown to function as an efficient gene vector in distantly related species.

MATERIALS AND METHODS

Plasmid constructions: Plasmid p*Hermes*Kan served as the *Hermes*-donor in the extra-chromosomal transpositional recombination assay. It was constructed by ligating a fragment from the B5 *Hermes* clone (WARREN *et al.* 1994) containing 1.4 kb of the right end of *Hermes* and a fragment from the E1 clone of *Hermes* containing 1.1 kb of the left end of *Hermes* (WARREN *et al.* 1994) into the polylinker of pBC(KS$^+$) (Stratagene). A 1.4-kb fragment from pACYC184 (CHANG and COHEN 1978) containing the kanamycin resistance gene was inserted into this plasmid between the termini, creating p*Hermes*Kan. The *Hermes* element on this plasmid was flanked by 1.9 kb of *M. domestica* genomic DNA at the right end and 0.8 kb of flanking *M. domestica* genomic DNA at the left end.

Plasmid pBS*Hermes-w*$^+$ was used as the *Hermes* donor in germline transformation experiments in *D. melanogaster*. The

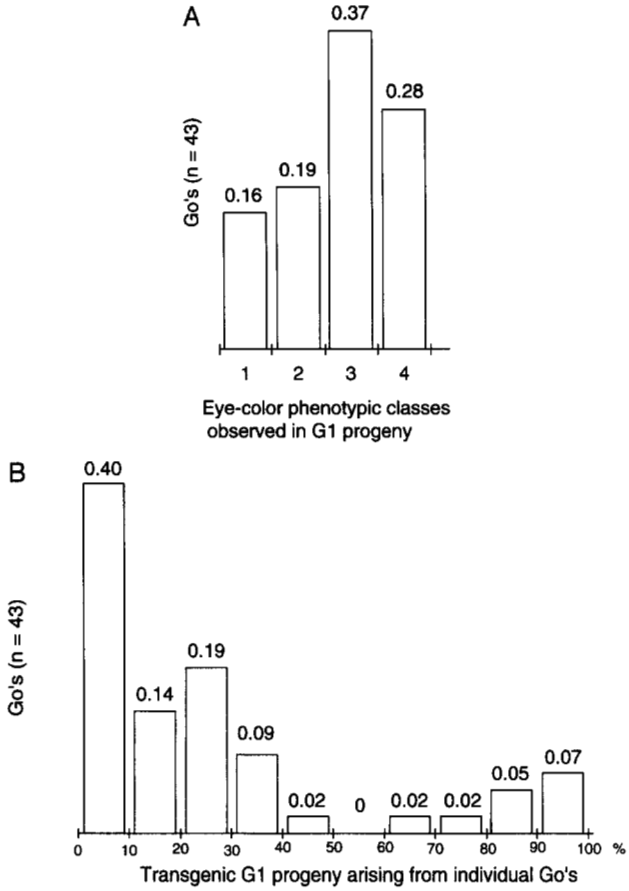

FIGURE 2.—Analysis of multiple germline insertions of *Hermes-w*$^+$ in G_0 adults. The progeny of G_0 adults with pigmented eyes were classified into four phenotypic classes (apricot, peach, crimson and red). (A) The proportion of G_0 adults with transgenic germlines yielding progeny in 1, 2, 3, or all four phenotypic classes. (B) The proportion of transgenic progeny with pigmented eyes arising from G_0s with transgenic germlines. An average of 184 ± 59 progeny from individual G_0s were scored for w^+ expression.

right and left ends of *Hermes* with flanking *M. domestica* genomic DNA, described above, were inserted into pBS(KS$^-$) (Stratagene). A 3.6-kb fragment containing the *Drosophila* mini-*white* gene (PIRROTTA 1988) was inserted between the right and left termini of *Hermes* to create pBS*Hermes-w*$^+$.

Plasmid pHSHH1.9 served as a source of *Hermes* transposase in germline transformation and somatic remobilization experiments. This plasmid was constructed by isolating an intact *Hermes* transposase open reading frame from *M. domestica* genomic DNA using PCR. Using high fidelity UlTma DNA polymerase (Perkin-Elmer) and genomic DNA from the Maryland strain of *M. domestica* (ATKINSON *et al.* 1993), a 1.9-kb PCR product containing sequences from nucleotides 425 to 2381 of the published *Hermes* sequence (Genbank accession number L34807) was inserted into pBC(KS$^+$) (Stratagene) to yield pHH1.9. The presence of an intact open reading frame was confirmed by DNA sequencing. The 1.9-kb *Hermes* transposase ORF was transferred to the plasmid pHSREM2, placing the ORF 3′ of the *D. melanogaster hsp70* promoter and 5′ of a concensus polyadenylation signal (KNIPPLE and MARSELLA-HERRICK 1988) to create pHSHH1.9.

Plasmid pBCHSHH1.9 served as a source of *Hermes* transposase in the extra-chromosomal transpositional recombination assay. This *Hermes* transposase helper plasmid was constructed by removing the *hsp70*-transposase ORF expression cassette from pHSHH1.9 and transferring it to pBC(KS$^+$) (Stratagene) to create pBCHSHH1.9.

Plasmid pUCSacRB, containing the sucrase gene from *B. subtilis*, was constructed previously and served as the target plasmid in the interplasmid transposition assay (O'BROCHTA *et al.* 1994).

Extrachromosomal *Hermes* transposition assay: Extra-chromosomal transpositional recombination of *Hermes* was assessed using the plasmid assay system described by O'BROCHTA *et al.* (1994) with the following modifications (Figure 1A). A mixture of CsCl-purified plasmid DNA consisting of pBCHSHH1.9 (0.5 mg/ml), p*Hermes*Kan (1 mg/ml) and pUCsacRB (1 mg/ml) was injected into preblastoderm *M. domestica* embryos (O'BROCHTA *et al.* 1994). Plasmid DNA was recovered 6–12 hr post injection, electrophorated into *E. coli* strain DH12S (GibcoBRL) and selected on LB agar plates containing ampicillin (0.1 mg/ml), kanamycin (0.05 mg/ml) and sucrose (10%). Plasmid DNA prepared from amp^r/kan^r/suc^r colonies was mapped with restriction endonucleases and the DNA sequence at the junction between *Hermes* and *sacRB* determined using *Hermes*-specific oligonucleotide primers (SANGER *et al.* 1977).

Germline transformation of *D. melanogaster*: Preblastoderm embryos of the *D. melanogaster* strain yw^{67c23} (obtained from Dr. B. CALVI, Dept. of Embryology, Carnegie Institute of Washington), which lacks both *hobo* and *P* elements, were injected with a mixture of p*Hermes-w*$^+$ (0.5 mg/ml) and pHSHH1.9 (0.5 mg/ml) using standard procedures (SPRADLING 1986). G_0 adults were backcrossed to yw^{67c23} and G_1 progeny with pigmented eyes recovered. Integrated *Hermes-w*$^+$ elements from selected pigmented G_1 individuals were mapped and homozygous lines established.

Southern and *in situ* hybridizations: Southern blot analysis of genomic DNA was performed at high stringency as described (KREITMAN and AGUADÉ 1986) and probed with a 4-kb fragment isolated containing *Hermes* left and right ends plus flanking *M. domestica* genomic DNA and labeled with ^{32}P using random primers. For *in situ* nick translated *Hermes* DNA was labeled with biotinylated dUTP, hybridized to late third instar salivary gland chromosomes (ASHBURNER 1989) and detected using the DETEK Hrp Signal generating system (Enzo Diagnostics) in accordance with the manufacturers recommendations.

Inverse PCR: Approximately one fly equivalent of genomic DNA was digested to completion with *Sau*3A and circularized by ligation in a volume of 200 μl. Ligated DNA was purified by GENECLEAN (Bio101) and recovered in 20 μl of water. One microliter of circularized DNA was used as template in a 40 μl PCR containing 2.5 mM $MgCl_2$, 0.5 μM of each primer, 200 μM dNTPs, 50 mM KCl, 10 mM Tris-HCl (pH 8.3) and 2.5 U AmpliTaq DNA polymerase (Perkin-Elmer). Oligonucleotide primers 5′-AATGAATTTTTTGTTCAAGTGGCAAG-CAC-3′ and 5′-CAGTCGCCTGCCTTATGCTTTTGGAGA-GCG-3′ were used to amplify the left terminus of *Hermes* and primers 5′-GAGTATTTTTTCACAACTTAACAACAACAG-3′ and 5′-AAAATACTTGCACTCAAAAGGCTTGACACC-3′ were used to amplify the right terminus of *Hermes* from circular templates. Reaction temperature conditions were: 95° (3 min), followed by 30 cycles of 95° (15 sec), 65° (15 sec), 72° (60 sec), followed by 3 min at 72°. PCR products were reamplified under identical conditions, cloned into TA Cloning vectors (Invitrogen) and sequenced (SANGER *et al.* 1977).

Remobilization of integrated *Hermes* elements: Preblastoderm embryos were collected from transgenic line 509, dechorionated and microinjected with pHSHH1.9 transposase helper plasmid using standard procedures (SPRADLING 1986). The DNA was injected at a concentration of 0.25 mg/ml into the anterior dorsal region of the embryo resulting in the deposition of DNA in the region of the blastoderm where eye

TABLE 1

Genetic analysis of the progeny from a single G_0 individual with a transgenic germline

Apricot (Σ = 39)			Peach (Σ = 33)			Crimson (Σ = 30)			Red (Σ = 4)			White (Σ = 141)
G_1	G_2	Chromosome	G_1	G_2	Chromosome	G_1	G_2	Chromosome	G_1	G_2	Chromosome	
1	ap	*X*	4	pch	*3*	7	red	*3*	11	red	*2*	
							ap	ND[a]				
2	ap	*2*	5	pch	*X*	8	red	*3*				
							ap	*X*				
3	ap	*X*	6	pch	*2*	9	red	*3*				
							ap	*X*				
						10	red	*3*				
							ap	*X*				

The G_1 progeny from a single G_0 individual with a transgenic germline were assigned to one of five phenotypic classes based on eye color: apricot (ap), peach (pch), crimson, red and white. The number of progeny assigned to each of the classes is indicated by Σ. Eleven male progeny (G_1) with pigmented eyes were used to map the locations of the integrated *Hermes* elements. The phenotypes of the G_2 progeny arising from the transgenic G_1 individuals are indicated. The chromosomal location of the integrated *Hermes* elements, as determined by genetic mapping, are indicated (*X*, *2*, and *3* are chromosome designations).

[a] Not determined.

imaginal disc cells are determined. Transient expression of *Hermes*-transposase and mobilization of *Hermes-w*$^+$ elements in eye imaginal disc cells resulted in clones of ommatidia with altered pigmentation in adult males developing from the injected embryos.

RESULTS

***Hermes* transposition in *M. domestica*:** We tested the ability of *Hermes* to transpose in its host, *M. domestica*, using an *in vivo* transient expression system combined with a method for detecting interplasmid transpositional recombination (Figure 1A) (O'BROCHTA *et al.* 1994). Of ~10^6 target plasmids recovered and screened, two harbored insertions of the *Hermes* element. These insertions involved only sequences precisely delimited by the terminal inverted repeats of the *Hermes* element and the integrated elements were flanked by 8 bp of direct sequence duplications of the insertion site (Figure 1B). These features are hallmarks of transpositional recombination indicating that these insertions resulted from genuine *Hermes* transposase-mediated transposition reactions. The frequency with which *Hermes* transposition events were recovered from *M. domestica* was ~10-fold lower than the frequency with which *hobo* transposition events were recovered under similar conditions using an analogous assay system in *D. melanogaster* (O'BROCHTA *et al.* 1994). However, the *Hermes* transposition recovery rate in *M. domestica* was only 2.5-fold lower than that observed using the *hobo* element in *M. domestica* (O'BROCHTA *et al.* 1994). Control experiments in which the same mixture of transposition-assay plasmids was transformed directly into *E. coli* failed to recover any *Hermes* insertions into *sacRB* after screening ~10^7 target plasmids.

***Hermes* transposition in *D. melanogaster*:** We assessed the ability of *Hermes* to act as a gene vector in *D. melanogaster*, an insect species distantly related to *M. domestica*, by using it to mediate germline transformation. Plasmids p*Hermes-w*$^+$ and pHSHH1.9 were coinjected into the posterior pole of preblastoderm *yw*^{67c23} *D. melanogaster* embryos. From three experiments, 124 fertile G_0 adults were recovered and individually backcrossed to *yw*^{67c23}. G_1 progeny with pigmented eyes were recovered from 43 (34.6%) of the 124 lines tested, indicating that *Hermes-w*$^+$ was transmitted through the germline of the G_0 generation. The proportion of G_0 adults producing transgenic offspring is comparable with that observed using *P* or *hobo* element-based vectors in *D. melanogaster* (SPRADLING 1986; BLACKMAN *et al.* 1989).

The *w*$^+$ progeny of G_0 individuals with transgenic germlines were classified into four phenotypic classes based on eye color (apricot, peach, crimson and red). Of the 43 G_0 individuals that produced transgenic progeny, 36 (83.7%) produced progeny with two or more distinct *w*$^+$ eye-color phenotypes (Figure 2A). Variation in the level of eye pigmentation is commonly observed with *P* element transformation vectors containing the mini-*white* gene and can be attributed to the position-dependent expression of the *w*$^+$ transgene (PIRROTTA 1988). Thus, G_0 insects producing progeny with a variety of eye-color phenotypes suggested that multiple integrations of *Hermes-w*$^+$ occurred in some G_0 germlines. Multiple integrations of *hobo*- and *P*-element gene vectors into individual germlines of *D. melanogaster* have been reported but typically at a lower frequency (SPRADLING 1986; BLACKMAN *et al.* 1989). Segregation analysis of 11 selected G_1 male offspring from the same G_0 individual confirmed that G_0s producing progeny with a variety of eye-color phenotypes had multiple germline integrations of *Hermes-w*$^+$ (Table 1). Thus Hermes appears to be capable of high levels of activity in *D. melanogaster* germ cells.

Of the 43 G_0 individuals with transgenic germlines, 60% produced clusters of transgenic progeny (Figure 2B), where a cluster was defined as a group of G_1 progeny with pigmented eyes that comprised ≥10% of the progeny arising from a single G_0 individual. In 7% of

the cases where clusters of G_1 progeny were observed, the cluster comprised ≥90% of the total G_1 progeny (Figure 2B). When *P* elements were used as vectors, SPRADLING (1986) reported 36.2% of 83 transgenic G_0 germlines producted clusters of transgenic progeny. Frequent clustering and large cluster sizes may reflect *Hermes-w*$^+$ hyperactivity and/or the integration of *Hermes-w*$^+$ early in the development of the germline of injected individuals.

Several transgenic lines were established from single male progeny of individual G_0 individuals with transgenic germlines. Integration of the *Hermes-w*$^+$ element into the genomic DNA of these lines was confirmed by Southern blot analysis (Figure 3), *in situ* hybridization analysis of polytene chromosomes (data not shown) and DNA sequence analysis of genomic sequences flanking integrated *Hermes-w*$^+$ elements (Figure 4). As predicted from genetic mapping, some lines contained multiple integrations of *Hermes-w*$^+$, with one line (#507) having ≥10 integrated *Hermes* elements (Figure 3). Integration of *Hermes-w*$^+$ involved only sequences precisely delimited by the element's terminal inverted repeats and resulted in the duplication of eight bases at the integration site, confirming that chromosomal integration was driven by transpositional recombination (Figure 4). The consensus sequence of integration sites of *Hermes-w*$^+$ [ATA($^T/_C$)TAAC] was remarkably similar to the consensus integration site of *hobo* elements in *D. melanogaster* (CTTTCAAC), suggesting that these two *hAT* elements have similar insertion-site preferences in this species (STRECK *et al.* 1986).

Integrated *Hermes-w*$^+$ elements were stable in the absence of *Hermes* transposase but remained capable of remobilization if *Hermes* transposase was reintroduced. Remobilization of *Hermes-w*$^+$ was observed after injecting the pHSHH1.9 transposase-helper plasmid into preblastoderm embryos of line 509, which contains a single, *X*-linked *Hermes-w*$^+$ element. Transient expression of *Hermes* transposase in early imaginal disc cells resulted in clones of ommatidia displaying altered pigmentation patterns in the eyes of 80% of the adult males recovered (Figure 5). Clones of ommatidia with altered pigmentation patterns were not observed in the absence of injected transposase helper plasmids. Both clones of ommatidia with reduced pigmentation levels and clones with darker levels of pigmentation were observed in the presence of helper plasmid. The darker clones of ommatidia were most likely caused by the transposition of the *Hermes-w*$^+$ to a new chromosomal location. Clones of ommatidia with reduced pigmentation were most likely the result of excision and loss of the *Hermes-w*$^+$ element or transposition of the element to a new chromosomal location that reduced or prevented mini-*white* expression. Somatic mobilization (excision and transposition) of *P* elements containing the mini-*white* gene resulted in similar phenotypes (LASKI *et al.* 1986).

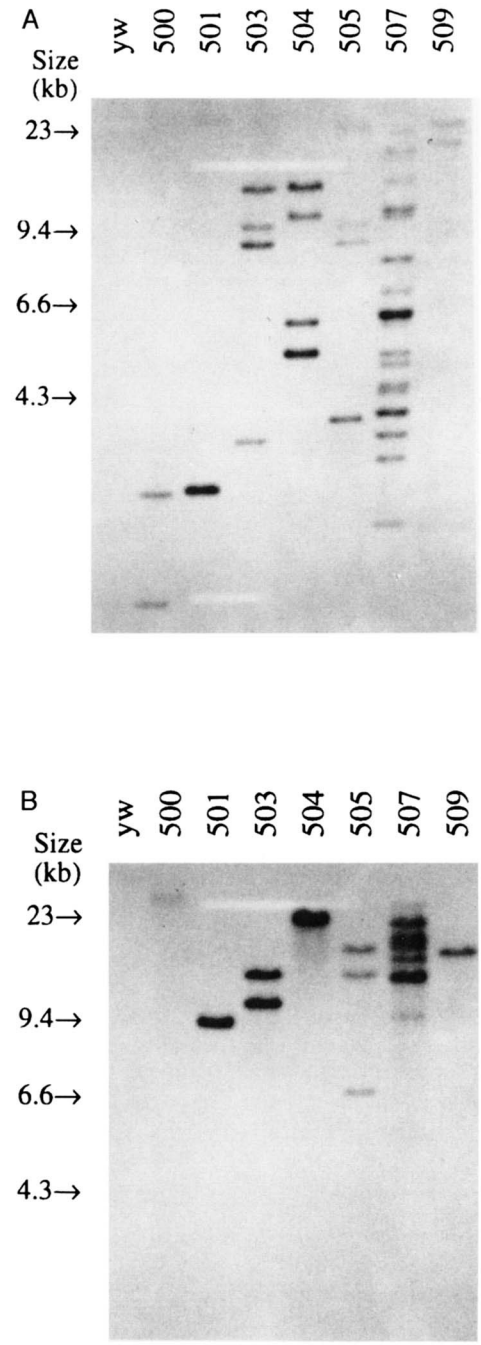

FIGURE 3.—Genomic Southern blot analysis of *D. melanogaster* lines containing the *Hermes-w*$^+$ transgene. The lines shown were derived from independent transgenic G_0 germlines. Genomic DNA (~4 μg) from a number of different lines was digested with *Eco*RI (A) or *Bam*HI (B) and probed with a *Hermes*-specific probe. *Bam*HI digests will generate a single unique band of hybridization for each *Hermes-w*$^+$ insertion present in the genome because there are no *Bam*HI sites within the element. Digestion with *Eco*RI will generate two unique bands of hybridization for each *Hermes-w*$^+$ insertion due to the presence of internal *Eco*RI sites flanking the *w*$^+$ marker. Hybridization patterns suggest that lines 500, 501 and 509 each contain a single element insertion, 503 and 504 contain two insertions, 505 contains three insertions and 507 contains ~10 independent insertions of *Hermes-w*$^+$. Lines 501, 503, 504, 505 and 509 were established from sibling G_1 individuals. Lines 500 and 507 were established from the progeny of different G_0s.

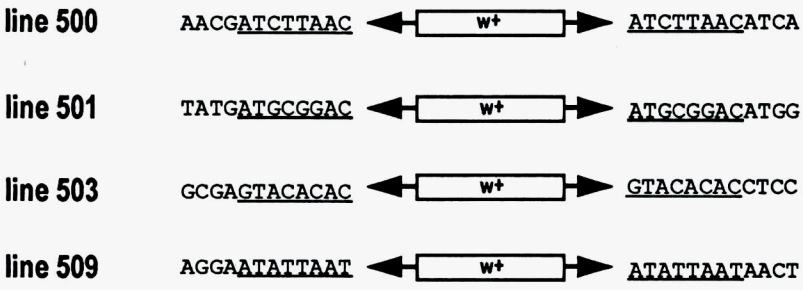

FIGURE 4.—Sequence analysis of *Hermes-w*$^+$ insertions. Sequence of the genomic DNA flanking the integrated *Hermes-w*$^+$ elements from four transgenic lines. The *Hermes-w*$^+$ sequences are shown diagrammatically in relation to the 8-bp duplication of the integration site (underlined). Only one of the two elements in line 503 was analyzed.

DISCUSSION

The presence of a *hobo*-like transposase activity in *M. domestica* was originally inferred from the observation that *hobo* elements could excise from plasmids introduced into *M. domestica* embryonic cells when *hobo* transposase had not been provided experimentally (ATKINSON *et al.* 1993). *M. domestica* was subsequently shown to contain a transposable element system, *Hermes,* that closely resembled the *hobo* element system (WARREN *et al.* 1994). Structural criteria, such as the presence of intact terminal inverted repeats, an open reading frame with the potential for encoding a protein with a high degree of similarity to the *hobo* transposase ORF and interstrain polymorphisms in copy number and insertion sites suggested that *Hermes* was a functioning transposable element system. Here we reported direct evidence confirming that *Hermes* elements can transpose in *M. domestica* and in the distantly related species *D. melanogaster.*

In *M. domestica* embryos *Hermes* elements accurately transposed between plasmids maintained extrachromosomally. These results clearly demonstrate that *Hermes* is capable of acting as a gene-vector, *i.e.,* promoting integration of nonelement sequences into DNA, in its natural host, *M. domestica.* Although *Hermes* transposition was infrequent in this species the rate was only 2.5-fold lower than that observed for *hobo* in *M. domestica* embryos under similar assay conditions (O'BROCHTA *et al.* 1994). As cells harboring many copies of a transposable element often have specific mechanisms for regulating their mobility, it is possible that the low rate of *Hermes* transposition we observed in *M. domestica* embryos resulted from an endogenous repression system. For example, when plasmid-based *P*-element excision assays were performed in *D. melanogaster* strains possessing numerous *P* elements, the frequency of *P* excision was reduced relative to that observed when strains lacking *P* elements were used (O'BROCHTA and HANDLER 1988). In *D. melanogaster, P*-element regulation is mediated in part by the presence of specific mutant forms of *P* elements that synthesize modified transposase-like proteins that interfere with the transposition process (RIO 1991). *M. domestica* strains are known to be polymorphic in copy number and structure of *Hermes* elements. The strain used in the experiments reported here contains ~20–30 copies of the *Hermes* (ATKINSON *et al.* 1993; WARREN *et al.* 1994) and while some of these endogenous elements appear intact, many contain internal deletions of the transposase coding region (WARREN *et al.* 1994). Although further experimentation will be required to establish if *Hermes* movement is regulated by modified *Hermes* elements, it could be advantageous to identify strains of *M. domestica* that lack *Hermes* elements to maximize the utility of *Hermes* based gene-vectors in this species. Our data provide strong evidence that *Hermes*-based vectors will be capable of mediating germline transformation of *M. domestica* and related species. *Hermes* is the first example of a transposable element isolated from a non-drosophilid insect that is functional and capable of serving as a gene-vector in a distantly related species. The development of suitable genetic markers and host strains should allow *Musca* transformation to become routine.

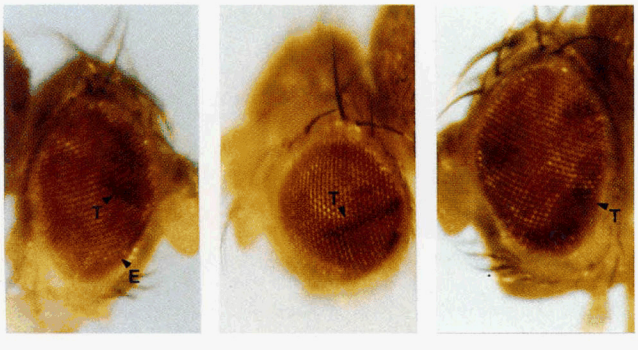

FIGURE 5.—Mosaicism resulting from somatic remobilization of an integrated *Hermes-w*$^+$ element. Shown are the eyes of three male progeny from line 509 that were injected at the preblastoderm stage with a plasmid (pHSHH1.9) expressing *Hermes* transposase. Clones of cells in which the *Hermes-w*$^+$ element excised (E) or transposed (T) are indicated.

The germline transformation of *D. melanogaster* using a *Hermes*-based gene-vector clearly demonstrated the ability of *Hermes* elements to function in an insect species distantly related to *M. domestica.* These data are notable for a number of reasons. First, *M. domestica* and *D. melanogaster* are members of different families of diptera and are thought to have diverged ~100 mya (HENNIG 1981). This indicates that *Hermes* either does not require host encoded factors for mobility, or that essential host-encoded factors are highly conserved. In either case our results indicate that *Hermes* is likely to have a broad host range. We are presently assessing *Hermes* mobility in a number of other insect species and have preliminary evidence indicating that *Hermes* can function in *Cochliomyia macellaria* (family, Calliphoridae), *Lucilia cuprina* (family, Calliphoridae), *Bactrocera*

tryoni, (family, Tephritidae) and *Aedes aegypti* (family, Culicidae) (A. SAKAR, J. SCURA, C. COATES, C. YARDLY, A. JAMES, P. ATKINSON and D. O'BROCHTA, unpublished data). Our results indicate that *Hermes'* ability to transform *D. melanogaster* is unlikely to be due to a unique characteristic of this species. The broad host range exhibited by other *hAT* elements suggests that *Hermes*-based gene-vectors will prove useful in a large number of insects of medical and agricultural importance.

Another notable feature of the data reported here is the frequency with which *Hermes*-mediated transformants were recovered in *D. melanogaster.* Both *P* and *hobo* elements had decreased levels of activity when introduced into species other than their natural host (O'BROCHTA and HANDLER 1988; O'BROCHTA *et al.* 1994), however, this appears not to be the case for *Hermes.* The mobility characteristics of *Hermes* are comparable to the most active transposable elements know to act as gene-vectors in *D. melanogaster.* Multiple element insertions and large clusters of transgenics are observed with *P* and *hobo*-element vectors (SPRADLING 1986; BLACKMAN and GELBART 1989), but they typically do not occur with the frequency seen using *Hermes.* If the rate of chromosomal integration reported here proves to be a general property of *Hermes* then *Hermes*-based vectors will be useful for both Drosophila and non-drosophilid insect scientists. Factors that may affect *Hermes* mobility, such as the influence of insert size on mobility and the possibility of interactions with endogenous transposable elements, are currently being investigated.

Based on our initial analysis of integration sites, *Hermes* elements appear to favor sequences resembling those preferred by *hobo.* These similarities further underscore the similarities between these two elements that have already been shown to have similar inverted repeats and transposase coding regions (WARREN *et al.* 1994). While these data suggest that *Hermes* and *hobo* may have a similar chromosomal targeting profiles, the elevated activity of *Hermes* may permit its use as a gene tagging agent for regions of the Drosophila genome that are poor targets for *P* elements.

The development of a functional non-drosophilid insect gene vector is an encouraging advance in the field of insect molecular science. Harnessing the *Hermes* transposable element and vectors derived from it will permit the implementation of powerful genetic research programs to investigate the biology of insects of significance to human health and welfare. In addition, insect population control strategies that require sophisticated and specialized methodologies of genotype manipulation should become possible. The development of these genotype manipulation technologies for insects will also be instrumental in developing analogous technologies for other arthropods and invertebrates, particularly those that are important to modern aquaculture industries.

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