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## Patterns of *Hermes* transposition in *Drosophila melanogaster*

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**Abstract** Transposable elements are being developed as tools for genomics and for the manipulation of insect genotypes for the purposes of biological control. An understanding of their transposition behavior will facilitate the use of these elements. The behavior of an autonomous *Hermes* transposable element from *Musca domestica* in the soma and germ-line of *Drosophila melanogaster* was investigated using the method of transposon display. In the germ-line, *Hermes* transposed at a rate of approximately 0.03 jumps per element per generation. Within the soma *Hermes* exhibited markedly non-random patterns of integration. Certain regions of the genome were distinctly preferred over others as integration targets, while other regions were underrepresented among the integration sites used. One particular site accounted for 4.4% of the transpositions recovered in this experiment, all of which were located within a 2.5-kb region of the *actin5C* promoter. This region was also present within the *Hermes* element itself, suggesting that this clustering is an example of transposable element "homing". Clusters of integration sites were also observed near the original donor sites; these represent examples of local hopping. The information content (sequence specificity) of the 8-bp target site was low, and the consensus

target site resembles that determined from plasmid-based integration assays.

**Keywords** Transposable elements · *Hermes* · *P*-elements · Local hopping · Homing

### Introduction

Transposable elements continue to hold the attention of geneticists and biologists because of their interesting genetic properties and characteristics within individuals and populations. The ability of transposons to significantly influence the genotype and phenotype of host organisms is often a direct consequence of an element's movement. The use of transposable elements for gene identification and analysis in *Drosophila melanogaster* has served as a paradigm, illustrating their potential to be developed into a variety of powerful genomics tools. More recently the range of organisms in which transposable element-based genomics tools are being developed has increased significantly. The successful reconstruction of the fish transposable element *Sleeping Beauty* and its use in the creation of transgenic fish and mice is one specific recent example (Fischer et al. 2001; Horie et al. 2001) of the development of such transposable element-based tools. Similar systems for use in plant, nematode and microbial systems also exist (Long et al. 1997; Chin et al. 1999; Goryshin et al. 2000; Bessereau et al. 2001). Insect biologists studying insects other than *Drosophila* have had a long-standing interest in developing transposable element-based tools, and transgenic insects have been created in a number of non-drosophilid species, using one of four gene vectors developed from distinct insect transposable elements (Atkinson and O'Brochta 1999; Atkinson et al. 2001). In the case of non-drosophilid insects, transposable elements are not only being employed as tools for manipulating insects in the laboratory, but are also being considered for use in altering the genotype of insects in natural populations. One particularly ambitious

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idea is to use transposable elements to spread a transgene through a natural population following an initial release of insects with genotypes created in the laboratory (Curtis 1994; Curtis and Graves 1988). Among those interested in this idea are mosquito biologists, who have considered introducing transgenes that render host insects incapable of transmitting pathogens. The human malaria vector *Anopheles gambiae* is one possible target for this form of genetic control (Collins and Besansky 1994; Collins 1994).

Based on our understanding of the natural history of a number of transposable elements, it should be possible to use transposable elements as agents to spread transgenes through natural populations. The almost ubiquitous distribution of *P*-elements in populations of *D. melanogaster* worldwide is thought to be the consequence of transfer of the element from the closely related species *D. willistoni* during the early part of the 20th century (Kidwell 1992). Once introduced into *D. melanogaster*, the element was capable of continued transposition and did so in a manner that resulted in its rapid spread to other chromosomes, enhancing its subsequent vertical transmission. Within less than a century almost all wild *D. melanogaster* populations had been invaded by *P* elements. That scenario seems to have been repeated multiple times in a number of *Drosophila* species (Silva and Kidwell 2000). The *mariner* element is a member of a large group of related elements that also appears to have undergone frequent horizontal transfer and spread widely, like the *P* element. However, in the case of some *mariner*-like elements horizontal transfer has apparently occurred between organisms from widely divergent phylogenetic groups (Robertson 1993; Robertson and Lampe 1995). Experimental efforts to recreate the phenomenon of transposable element spread in the laboratory have had some success, and illustrate the ability of some transposable elements to spread through small caged populations under some conditions (Good et al. 1989; Preston and Engels 1989; Meister and Grigliatti 1993; Kimura and Kidwell 1994; Ladeveze et al. 1994, 1998; Galindo et al. 1995; Carareto et al. 1997). However, as yet, the key characteristics that determine whether an element can successfully invade a new host have not been identified.

*Hermes* is a short inverted-repeat type element isolated originally from the house fly *Musca domestica* (Family Muscidae) (Warren et al. 1994). It is related to the *hobo* element from *D. melanogaster* and is a member of the *hAT* (*hobo*, *Ac*, *Tam3*) family of elements. It is approximately 3 kb in length with 17-bp imperfect inverted terminal repeats (ITRs) and an 1800-bp ORF encoding the *Hermes* transposase (Warren et al. 1994). Host range studies have shown that *Hermes* is capable of transposing in at least 15 insect species including mosquitoes, tephritid fruit flies, beetles and moths (Atkinson et al. 2001). These studies indicate that *Hermes* has the potential to be a useful tool for genetically manipulating non-drosophilid insects. The range and effectiveness of tools that may be developed from *Hermes* elements will be determined, in part, by the mobility characteristics of the element. Here

we report the results of an investigation of the transposition characteristics of an autonomous *Hermes* element in a foreign host. These and additional studies will be necessary to enable the efficient and effective use of *Hermes* in the laboratory as a tool for gene discovery and analysis, and as a means of genetically infiltrating natural populations.

## Materials and methods

### The autonomous *Hermes* element

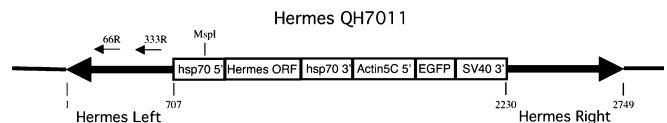
Plasmid pQH7011 contains the autonomous *Hermes* element QH7011. Autonomous transposable elements have functional ITR sequences and are capable of expressing functional transposase. pQH7011 was constructed by inserting a 2.3-kb *XhoI-XbaI* fragment containing the ORF for the *Hermes* transposase under the regulatory control of the *D. melanogaster hsp70* promoter from the plasmid pSHH1.9 (O'Brochta et al. 1996) into the blunt-ended *EcoRI* site of the plasmid pHermes(A5CEGFP) (Pinkerton et al. 2000) (Fig. 1).

### Construction of transgenic *Drosophila* strains

Transgenic *D. melanogaster* flies were created by injecting preblastoderm embryos from the strain *Canton S w* with a solution containing pQH7011 (0.5 mg/ml in 5 mM KCl, 0.1 mM NaPO<sub>4</sub> pH 6.8) following standard procedures (Rubin and Spradling 1982; Spradling and Rubin 1982). G<sub>0</sub> adults were backcrossed to *Canton S* individuals, and the resulting G<sub>1</sub> progeny were screened for expression of enhanced Green Fluorescent Protein (EGFP). Seven G<sub>0</sub> individuals produced transgenic progeny. These progeny were used to establish seven independent populations. An eighth population was established by pooling approximately ten individuals from each of the seven initial populations. Because the *Hermes* elements are autonomous and are capable of transposition, the establishment of stable homozygous lines is impossible. Consequently insects within a population will have a variety of genotypes. Individual males from these populations were used in subsequent test crosses.

### Crosses

Individual males from the QH7011 populations were each mated to five virgin *Canton S* females. Following mating and egg deposition the parental insects were frozen at -80°C. Progeny were collected as



**Fig. 1** The autonomous *Hermes* element QH7011 (not to scale). The large arrowheads represent the terminal inverted repeat sequences of *Hermes*. The numbers below the element refer to the nucleotide positions in an unaltered *Hermes* element, with the first nucleotide of the left inverted terminal repeat being nucleotide 1. The locations of the *Hermes*-specific primers are shown by the small arrows above the element. The internal *MspI* site that is closest to the left terminal inverted repeat is marked. The designations hsp70 5' and hsp70 3' refer to the promoter and 3' untranslated regions of the *D. melanogaster hsp70* gene, respectively. Hermes ORF is the transposase-coding region. Actin5C 5' is the distal promoter region of the cytoplasmic actin gene *actin5C*. EGFP is the ORF encoding of the enhanced Green Fluorescent Protein. SV40 3' is the 3' untranslated region from the large T antigen gene of the polyoma virus SV40

newly emerged adults and stored at  $-80^{\circ}\text{C}$  until needed. Insect were reared at room temperature ( $24^{\circ}\text{C}$ ) on standard (cornmeal-molasses) *Drosophila* medium. Because the elements used in this study were autonomous it was not possible to establish and maintain lines with stable genotypes. Consequently many crosses were performed and the progeny collected. Following genotyping of the parents, certain crosses were selected as being useful for addressing particular questions, and the progenies of these crosses were analyzed. Two types of crosses were of particular interest in this study: those in which it was important to start with males containing a single *Hermes* element (local hopping and homing analysis) and those in which it was important to start with males containing multiple elements (transposition rates).

### Transposon display

Transposon display is a DNA fingerprinting method that is similar to the Amplified Fragment Length Polymorphism (AFLP) method (Vos et al. 1995). AFLP relies on the chance annealing of short adapter-specific PCR primers to display a subset of genomic DNA fragments generated following restriction endonuclease digestion and ligation to specific adapters. Transposon display is similar but uses a slightly different set of PCR primers. One primer is specific to the transposable element of interest, while the second is an adapter-specific primer located outside the element. Use of this combination allows one to display a subset of genomic DNA fragments containing the ITR of the element and flanking genomic DNA sequences (Van den Broeck et al. 1998; Casa et al. 2000). The transposon display method permits the simultaneous detection of many instances of transposition of the same element in individual insects. Because the method is dependent upon the amplification of genomic DNA fragments using PCR, in the protocol described here large fragments ( $>1$  kb) tend to be under-represented.

Total genomic DNA was extracted from individual adult *D. melanogaster* using the Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's specifications. Genomic DNA from a single insect was resuspended in  $30\ \mu\text{l}$  of water.

Five microliters of genomic DNA (approximately 200 ng) was digested for 3 h in a volume of  $40\ \mu\text{l}$  at  $37^{\circ}\text{C}$  with 4 U of the restriction endonuclease *MspI* or *HpyCH4IV* under the reaction conditions recommended by the enzyme supplier (New England Biolabs). Sixty picomoles of adapters was ligated to the digested genomic DNA by adding  $10\ \mu\text{l}$  of the appropriate  $1\times$  restriction enzyme buffer containing 5 mM ATP, 50 mM DTT and  $10\ \mu\text{g}$  of BSA, and incubated at  $37^{\circ}\text{C}$  for 3–12 h in the presence of 1 Weiss unit of T4 DNA ligase. The adapters consisted of a duplex of the oligonucleotides *MspIa* ( $5'$ -GACGATGAGTCCTGAG- $3'$ ) and *MspIb* ( $5'$ -CGCTCAGGACTCAT- $3'$ ) and were compatible with *MspI*- and *HpyCH4IV*-digested genomic DNA. At the end of the ligation reaction the mixture was diluted by adding  $150\ \mu\text{l}$  of  $0.1\times\text{TE}$  ( $1\times\text{TE}$  contains 10 mM TRIS-HCl pH 7.6, 1 mM EDTA pH 8.0). For some of the experiments described below it was important to confirm that certain parental insects contained only a single germ-line integration of the QH7011 element. In these cases the insects were genotyped twice using transposon display. In one case the genomic DNA was digested with *MspI* and in the other with *HpyCH4IV*. By using two four-base-cutters to prepare the genomic DNA the possibility that a germ-line integrated element might go undetected because the genomic DNA fragment containing the end of *Hermes* was large and inefficiently amplified was minimized.

PCRs were performed in a  $50\text{-}\mu\text{l}$  reaction volume containing  $1\times$  PCR Buffer II (Applied Biosystems), 0.2 mM dNTPs (an equimolar mixture of dATP, dTTP, dCTP, dGTP), 2.5 mM  $\text{MgCl}_2$ , 1 U of AmpliTaq DNA polymerase (Applied Biosystems), 24 pmol of primer *MspIa* and primer *Hermes* 333R ( $5'$ -TCGGAA-CATTTTGCTGTG- $3'$ ) and  $5\ \mu\text{l}$  of the diluted restriction/ligation reaction. The DNA polymerase was added as a complex with TaqStart Antibody (Clontech) as described by the manufacturer for the purposes of hot-starting the reaction. Reactions were carried out in a Perkin-Elmer 9600 thermocycler using the following

parameters:  $95^{\circ}\text{C}$  for 5 min, followed by 25 cycles of  $95^{\circ}\text{C}$  for 15 s,  $61^{\circ}\text{C}$  for 1 min, and  $72^{\circ}\text{C}$  for 1.5 min, with a final incubation at  $72^{\circ}\text{C}$  for 5 min. Following the PCR  $5\ \mu\text{l}$  of the reaction mixture was diluted with  $95\ \mu\text{l}$  of  $0.1\times\text{TE}$

The second, selective, PCR was then performed using a *Hermes*-specific primer, *Hermes66R+* ( $5'$ -AATGAATTTTTTGTTCAGTGGCAAAGCAC- $3'$ ), that was labeled at the  $5'$  end with Cy5 (Amersham Life Science). Cy5-labeled primers were purified by HPLC prior to use. Using  $5\ \mu\text{l}$  of the diluted products of the first, preselective, PCR as a template, a  $20\text{-}\mu\text{l}$  PCR containing  $1\times$  PCR Buffer II, 0.2 mM dNTPs, 2.5 mM  $\text{MgCl}_2$ , 1 U of AmpliTaq DNA polymerase, 9 pmol each of primers *MspIa* and Cy5-*Hermes66R+* was set up. The reaction was initiated by incubation at  $95^{\circ}\text{C}$  for 3 min, followed by five cycles in which the annealing temperature was decreased  $1^{\circ}\text{C}$  after each cycle. The reaction parameters for the first of these cycles were:  $95^{\circ}\text{C}$  for 15 s,  $60^{\circ}\text{C}$  for 1 min, and  $72^{\circ}\text{C}$  for 1.5 min. Following these five cycles of "touchdown" PCR, 25 cycles were performed at  $95^{\circ}\text{C}$  for 15 s,  $56^{\circ}\text{C}$  for 1 min,  $72^{\circ}\text{C}$  for 1.5 min, with a final extension step at  $72^{\circ}\text{C}$  for 5 min.

A  $3\text{-}\mu\text{l}$  aliquot of the products of the selective PCR was mixed with  $3\ \mu\text{l}$  of loading buffer (95% deionized formamide, 10 mM EDTA), heated to  $95^{\circ}\text{C}$  for 5 min, cooled quickly on ice and loaded on a 6% polyacrylamide gel (19:1 acrylamide:bisacrylamide) containing 6.7 M urea in  $1\times\text{TBE}$  buffer (90 mM TRIS-borate, 2 mM EDTA). ALFexpress Sizer 50–500 (Amersham/Pharmacia) was used as a size standard. Electrophoresis was performed at constant power (70 W) for 4 h, at which time the gel was transferred to 3MM filter paper and dried. The dried gel was scanned on a STORM 860 phosphorimager (Molecular Dynamics).

An image of the gel was printed onto acetate film and overlaid on the dried gel. The area of the dried gel corresponding to the band of interest was excised and placed in  $100\ \mu\text{l}$  of water. A  $5\text{-}\mu\text{l}$  aliquot of the eluted sample was used as a template in a  $20\text{-}\mu\text{l}$  PCR using the same conditions as described for the selective PCR, except that unlabeled *Hermes66R+* was used. A  $10\text{-}\mu\text{l}$  sample of the reaction products was then sequenced using AmpliTaq DNA polymerase, FS and Big Dye terminators (Applied Biosystems) with dITP, and *Hermes66R+* as a primer. Reaction products were resolved on an Applied Biosystems DNA model 3100 sequencer.

DNA sequence information was used to perform a BLAST search of the *Drosophila* genome, either through the Berkeley *Drosophila* Genome Project (Fly BLAST, Release 2; <http://www.fruitfly.org/blast/>) or the National Center for Biotechnology Information (Nucleotide BLAST; <http://www.ncbi.nlm.nih.gov/BLAST/>) using the default parameters at both sites. Release 2 of the Genome Annotation Database of *Drosophila* was used for this analysis.

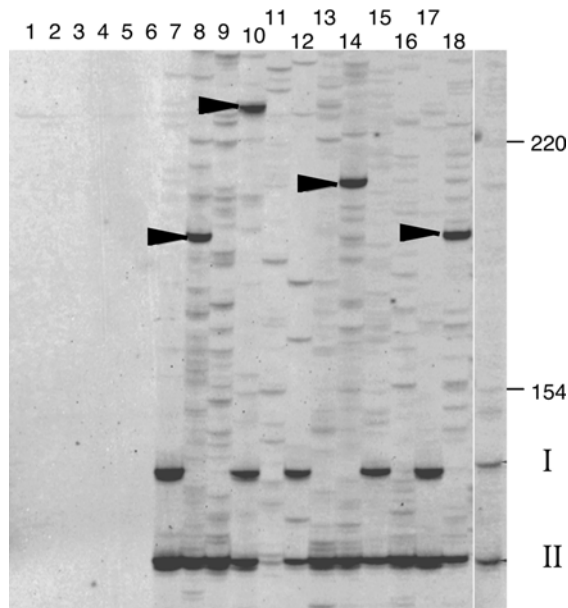
### Sequence logos

A sequence logo is a graphic representation of the amount of information to be found in a set of DNA, RNA or protein sequences (Schneider and Stephens 1990). A DNA sequence logo displays the general consensus sequence, the order of predominance of the nucleotides at each position, the relative frequencies of each nucleotide at each position and the amount of information present at every position in the sequence (measured in bits) (Schneider and Stephens 1990). We used *WebLogo*, a Web-based, interactive, multi-part script for generating sequence logos; it is available at <http://www.bio.cam.ac.uk/seqlogo/>.

## Results

### Germline and somatic transposition of *Hermes* QH7011

Transposon display analysis of individuals transformed with QH7011 revealed two classes of PCR products that differed in abundance (Fig. 2). The first were consis-



**Fig. 2** Transposon display of *Hermes* QH7011. Each numbered lane displays the *Hermes* elements contained in a single individual insect. Lanes 1–6 are non-transgenic Canton S female parents. Lanes 7–18 are transgenic progeny. The unlabelled lane on the right is the parental transgenic male. The Roman numerals I and II refer to *Hermes* elements present in the germ-line of the parental male. Note that this particular cross involved a parental male with multiple elements and was used to estimate transposition frequency. The positions of molecular weight markers (sizes are given in bp) are shown on the right. The arrowheads indicate transposition events that occurred in the germ-line of the parental male. The lighter bands derive from transposition events that occurred in the somatic tissue of the individual progeny

tently abundant products that arose from genomic DNA fragments that were well-represented in the initial pool of template molecules, and appear as dark bands following electrophoresis. These products are derived from templates that were transmitted vertically and represent germ-line integrations of QH7011. The second class consisted of PCR products of highly variable abundance, which were represented by bands that were moderately dark to faint on the transposon display gel. The number of these lighter bands observed in an individual could be high, and their patterns varied among individuals. These abundant, lighter bands did not arise from QH7011 elements that are transmitted vertically and cannot represent germ-line events. Instead, these products result from somatic transposition of QH7011 elements. The autonomous *Hermes* element QH7011 contains the *Hermes* transposase ORF under the regulatory control of the promoter from the *D. melanogaster hsp 70* gene. This promoter is known to be transcriptionally active in stressed and unstressed tissues of the soma and germ-line (Atkinson and O'Brochta 1992; Steller and Pirrotta 1986). We confirmed the somatic activity of the *Hermes* transposase in QH7011 by crossing individual QH7011 males to virgin females of a line of transgenic insects containing a single, homozygous copy of a non-autonomous *Hermes* element with

the mini-*white* gene of *D. melanogaster* in a *white* genetic background (O'Brochta et al. 1996). Heterozygous adult progeny had mosaic eyes, as a result of excision and transposition of *Hermes*::mini-*white* (data not shown). Hence, QH7011-containing individuals were mosaics consisting of clones of cells with unique genotypes as a result of somatic activity of the element during development.

To estimate germ-line transposition rates in insects containing QH7011, transgenic males were crossed individually to five virgin Canton S females. After 2 days the parental flies were removed and stored at  $-80^{\circ}\text{C}$  until needed. Progeny were permitted to develop into adults, at which time they were collected and stored at  $-80^{\circ}\text{C}$  until needed for analysis. The parental insects and 36 progeny from each of three crosses were analyzed by transposon display. For this analysis crosses involving QH7011 males with multiple germ-line copies of the autonomous element were selected. The males used for these crosses originated from a population of insects that had been derived from a mixture of individuals containing QH7011 in a variety of chromosomal locations. Many of the insects in this population had multiple copies of QH7011. Germ-line elements in the parental males were taken as being heterozygous if approximately half of the progeny received the element, and homozygous if essentially all of the progeny received the element (germ-line excisions may result in the loss of some elements). Some of the progeny had dark bands in new locations and these were interpreted as indicating transpositions of the QH7011 element in the germ-line of the parental male; such findings are consistent with our studies of vertical transmission. Rates of transposition were estimated by dividing the number of unique germ-line transposition events by the number of element-generations (Table 1). Bands shared by more than one progeny we only counted once. In this experiment we observed 14 transposition events in 576 element generations, which is equivalent to a transposition frequency of 2.4%.

#### Chromosomal distribution of *Hermes* QH7011

The high somatic activity of QH7011 provided us with a rich source of *Hermes* transposition events within the genome of *D. melanogaster*. We used this source of transposition events to investigate the behavior of the *Hermes* element within this genome. We successfully isolated, re-amplified and sequenced the products of 252 independent transposition events. Their sequences were used to search the *Drosophila* genome database using BLAST (Altschul et al. 1990).

The distribution of *Hermes* transposition events among chromosomes was related to the relative size of each chromosome. There are 120 Mb of euchromatic DNA in the genome of *D. melanogaster*, with the chromosomes X, 2L, 2R, 3L, 3R, and 4 comprising 18.2%, 19.2%, 17.9%, 20.4%, 23.4% and 1.0%, respectively,

**Table 1** Germline transposition of QH7011

Cross <sup>a</sup>	Elements <sup>b</sup>	Progeny	Element-generations	Transpositions	Frequency <sup>c</sup>
I	2	36	72	0	0%
II	8	36	288	12	4.1%
III	6	36	216	2	0.9%
Total			576	14	2.4%

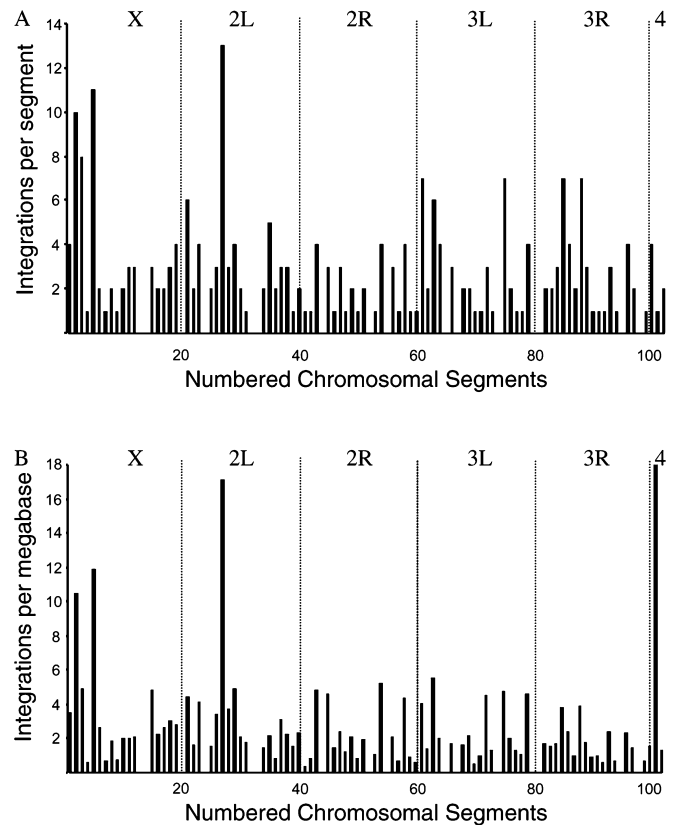
<sup>a</sup>All matings and progeny development occurred at 25°C

<sup>b</sup>Based on the frequency of progeny receiving parental elements we estimated that the parental males were homozygous for all of the elements tested

<sup>c</sup>Number of transpositions/number of element-generations

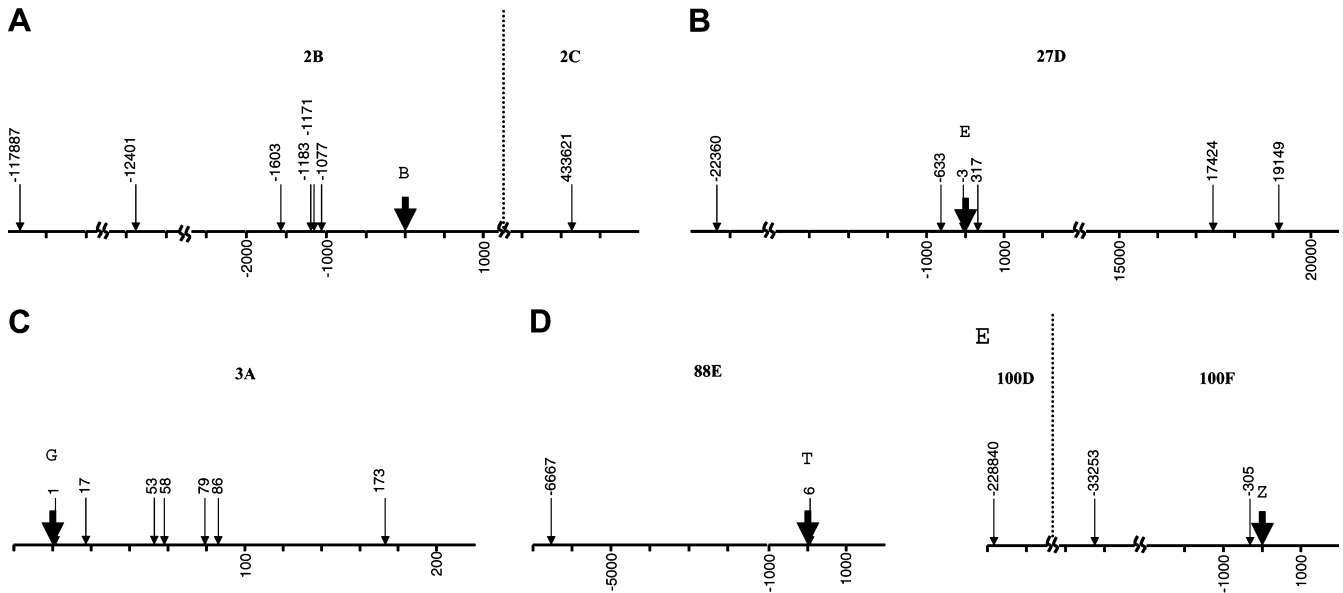
of the total. Of the 252 transpositions analyzed, 24.6%, 23.0%, 13.5%, 18.6%, 19.0% and 1.2% occurred on *X*, *2L*, *2R*, *3L*, *3R* and *4*, respectively. There is no evidence that the observed distribution of integrations differs significantly from that expected based on the size of each chromosome ( $X^2 = 12.70$ ;  $df = 5$ ;  $P = 0.25$ ). Determination of the number of transposition events in each numbered chromosomal region revealed a much more non-uniform distribution of *Hermes* elements, suggesting variation in the density of targets along the chromosomes. Sixteen of the 102 numbered chromosomal segments, which are based on polytene chromosome banding patterns, had not served as targets for *Hermes* transposition, while 10 segments had 6 or more *Hermes* integrations each (Fig. 3A). This pattern of transposition deviates significantly from that expected to arise from purely random integration of *Hermes* into the chromosomes based on a Chi-squared test comparing the observed distribution to a Poisson distribution of the same sample size. Normalizing these data to account for the actual size of each chromosomal segment did not substantially alter the pattern of integration, indicating that the number of integrations observed per chromosomal segment was not simply a function of the size of the segment (Fig. 3B). A closer examination of the data revealed at least three patterns of non-random integration.

First, clustering of integrations near the site of the original germ-line integration event (local hopping) was observed. Six crosses involving males containing a single and unique integration of QH7011 (see Materials and methods) provided the data described below. The six paternal QH7011 elements are referred to as elements B, D, E, G, T and Z. For five of the six elements analyzed (elements B, E, G, T, Z), multiple integrations ranging from two to seven events were recovered within the chromosomal segment containing the original germ-line integration. Element B was originally located at polytene chromosome position 2B4, and seven subsequent transpositions ( $n = 34$ ) were recovered in segment 2. Four of these new integrations were approximately 1 kb from the donor site (Fig. 4A). Element E started out in position 27D3, and six subsequent transpositions ( $n = 46$ ) were recovered in segment 27. Three of these integrations were located less than 1 kb from the donor site (Fig. 4B). Element G originated at position 3A2; seven subsequent transpositions ( $n = 27$ ) were recovered



**Fig. 3A, B** Frequency distribution of *Hermes* transposition as a function of numbered chromosomal segments. **A** Number of integrations per chromosomal segment. **B** The same data as in **A** but normalized to account for the amount of DNA present in each chromosomal segment. Note that segment 101 is currently estimated to contain only 25 kb of DNA, resulting in a very high estimated rate of integration in this region of the genome

in segment 3, and all were located less than 200 bp from the donor site (Fig. 4C). Element T originated at position 88E1 and two subsequent transpositions ( $n = 12$ ) were recovered in segment 88, each within 10 kb of the donor site (Fig. 4D). Element Z originated at position 100F1 and three subsequent transpositions ( $n = 30$ ) were recovered in segment 100 (Fig. 4E). The cytogenetic position of *Hermes* element D could not be determined definitively because it was located within the multicopy transposable element 297. Despite our inability to precisely locate the chromosomal position of element D, two remobilization events were also recovered within



**Fig. 4A–E** Locations of transposition events arising from local hopping. The location (*large arrow*) and name (*bold upper-case letter*) of the starting element are indicated. The *thin arrows* indicate *Hermes* insertion sites. The *numbers* refer to the distance (in bp) between the new integration site and the original insertion. Lettered polytene chromosome subdivisions are indicated. **A** Element B. **B** Element E. **C** Element G. **D** Element T. **E** Element Z

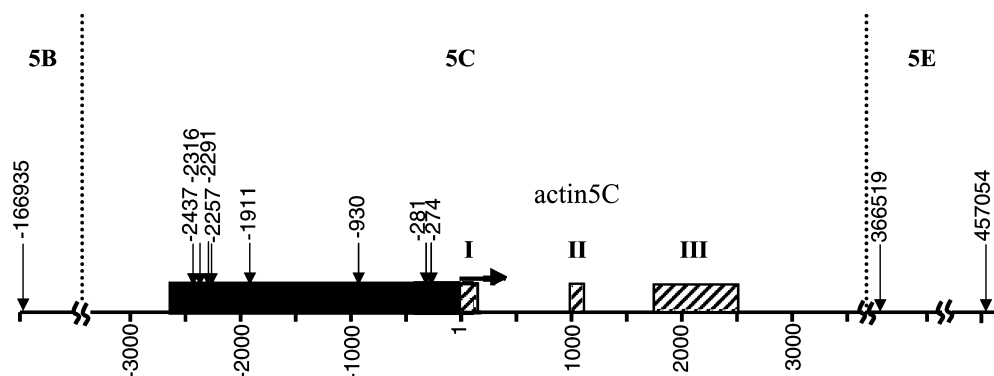
297 sequences—possibly as a result of local hopping from the original insertion site.

Secondly, non-random integration was observed as clusters of integration events at chromosomal positions that were often unlinked to the original germ-line integration site. For example, element B, originating at 2B4, was recovered three times each in segments 43 on 2R and 61 on 3L. Element E, originating at 27D3 on chromosome 2L, was recovered four times in segment 63 on chromosome 3L and three times in segment 85 on chromosome 3R. These unlinked clusters originating from a common germ-line integration event were not the result of secondary local hopping (bouncing) because the events were detected in independent progeny. In addition, polytene chromosome division 61 was also the site of four additional independent transposition events arising from elements G, D, E and Z. Polytene chromosome division 85 was the site of four additional independent transposition events arising from elements B, Z and D. Similarly, polytene chromosome divisions 75 and 88 were the targets of seven independent integrations arising from elements B, Z, D and T, respectively. Other examples of preferred regions of integration are polytene division 21 (five events recovered from three crosses), 27 (eight events recovered from three crosses), 35 (four events recovered from three crosses) and 96 (four events recovered from three crosses) (Fig. 3).

Finally, one particular gene was the target for multiple integrations of *Hermes*. The cytoplasmic actin gene located at polytene chromosome position 5C (*actin5C*) was the site of integration for 4.4% of the transpositions

recovered in this experiment. The integrations of *Hermes* into *actin5C* were located within a 2.5-kb region immediately upstream of the distal transcription start site (Fig. 5). These transposition events were all independent, and were recovered from individual progeny. What makes this clustering notable is the fact that the autonomous element used in this study contained a 2.5-kb segment of the distal promoter of *actin5C* that corresponded precisely to the chromosomal region containing the cluster of integration events (Fig. 1). While the parental males contained only a single germ-line copy of the QH7011 element, making it likely that the integrations recovered had resulted from transposition into the authentic chromosomal copy of the *actin5C* promoter region, we cannot rule out the possibility that some of these insertions actually occurred in the *actin5C* region contained within QH7011 itself. The latter would have arisen from secondary or tertiary transpositions, and while this is possible in some cases, it is unlikely to account for the majority. A clear pattern of local hopping in this experiment would not have been recognized if secondary and tertiary transposition events were prevalent. Whether or not the observed clustering was in the genomic *actin 5C* promoter or the sequences present on QH7011, the pattern itself is notable and may reflect an example of transposable element “homing”. Homing in this case refers to the non-random integration of a transposable element, mediated by sequences contained within the element that are homologous to sequences within the target genome.

While the number of polytene chromosomal segments with five or more integrated *Hermes* elements is greater than would be expected if integration were random (based on comparison with a Poisson distribution), segments that did not serve as a target for *Hermes* are also over-represented. Except for segments 80 and 81 on chromosome 3, all regions free of *Hermes* elements are well represented in the *Drosophila* sequence database.



**Fig. 5** Distribution of *Hermes* transpositions within polytene chromosomal division 5. Exons I, II and III of *actin5C* are depicted as *hatched boxes*. The transcriptional start site (+1) for the distal promoter is shown by the *horizontal arrow*. The distal promoter region corresponding to the region also found in the autonomous *Hermes* element QH701 is shown as a *black box*. This region extends from +8 to approximately -2700. The *numbers* refer to nucleotide position relative to the transcriptional start site. Lettered subdivisions within chromosomal segment 5 are shown

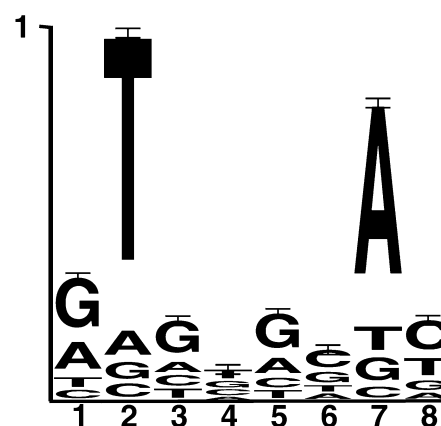
Segments 80 and 81 are centric regions of chromosome 3 for which there is little DNA sequence information and in which few genes have been identified. Consequently, failure to find integrations into this region does not necessarily reflect integration site preferences of *Hermes*.

#### Intragenic integration sites

Approximately 20% of the euchromatic sequences in the *D. melanogaster* genome are exons, 17% are intron sequences and the remaining euchromatic sequences consist of non-coding intergenic regions including 5' and 3' regulatory regions. Only 11% of the transposition events recovered in this experiment occurred into an exon of a known gene, while 39% of the events occurred in intron sequences. The remaining 50% were intergenic integrations. Twenty of the intergenic integrations were in regions of the chromosome for which there was enough experimental evidence to determine if the *Hermes* element was located in a 5' untranslated region (5' UTR) of a gene. Of these, only two (10%) were in known 5' UTRs, and from this we estimate that 5% of all *Hermes* integrations were into 5' UTRs.

#### Target site analysis

*Hermes* creates 8-bp direct duplications of the insertion site. We aligned 175 target-site duplications and used these data to produce a sequence logo to examine the quantity, quality and distribution of nucleotide sequence information within the site (Schneider and Stephens 1990) (Fig. 6). The overall information content of the target site was low, with less than 1 bit at each nucleotide position with 2 bits being the maximum for the complete sequence. Nucleotide positions 2 and 7 were the most



**Fig. 6** Sequence logo of the 8-bp target site duplications found in vivo. The *y*-axis shows 1 bit of information and the *x*-axis the nucleotide position within the target site

information-rich. Thymidine was the most common nucleotide at position 2 with 81% of the sites analyzed having this nucleotide. Three-quarters of the sites analyzed contained an adenine at position 7. Despite the low overall information content of the target site, the sequence logo reveals the presence of a near palindromic arrangement of nucleotides consisting of GTG at positions 1, 2 and 3 and CAC at positions 6, 7 and 8.

#### Discussion

We report the creation of an autonomous *Hermes* element carrying a dominant visible marker and have investigated its mobility characteristics within the genome of *D. melanogaster*. Understanding the behavior of transposable elements, particularly those being used or being considered for use as genomics tools, is critical for a number of reasons. First, many of the behaviors and characteristics of transposable elements are element-specific. Second, an understanding of the nature of these element-specific characteristics will be useful in assessing the appropriateness of a particular element for a particular application. Third, a thorough understanding of transposable element behavior will increase our ability to predict experimental outcomes. Finally, the predictive knowledge obtained through the investigation of

mobility characteristics will assist in assessing the risks associated with the use of transposable elements in applications involving the environmental release of transgenic organisms containing these elements.

Our measurements of mobility rates and characteristics relied on the transposon display technique. This AFLP-like method generates a fingerprint in the form of PCR products displayed following electrophoresis; the pattern obtained reflects both the number and location of target transposable elements. This method is conservative and tends to underestimate the number of elements in a genome, because efficient detection depends on the production of PCR products of approximately 1 kb or less. Because the preparation of genomic template DNA for subsequent PCR steps involves digestion with a restriction endonuclease, the absence of the appropriate restriction site within 1 kb of the terminus of a transposable element will result in that element being missed in this assay. For *D. melanogaster* the restriction endonuclease *MspI* cuts often and therefore provides an excellent source of appropriately sized genomic DNA fragments, minimizing, but not eliminating, the problem of underestimation of element numbers. Part of the analysis presented here depended upon the use of transgenic parental insects that contained only a single transmissible *Hermes* element. To ensure that parental insects did not contain multiple germ-line elements the insects were genotyped twice by transposon display using two restriction endonucleases with 4-bp recognition sites (*MseI* and *HpyCH4IV*) to prepare the template DNA (data not shown). This permitted individuals with multiple *Hermes* elements to be identified and excluded from that part of the analysis.

Our analysis of the distribution of *Hermes* integration events revealed that the euchromatic regions of all the chromosomes were approximately equally good targets for *Hermes*. Integration frequencies were not markedly over- or under-represented for any chromosome or chromosome arm. However, when the distribution of integrations was examined as a function of numbered polytene chromosome divisions and nucleotide position within the genome, a number of patterns emerged that reflected *Hermes*-specific mobility properties. *Hermes* displayed a non-random distribution within the genome; the majority of polytene chromosome divisions never experienced *Hermes* integration (0 class), while others were targeted five or more times (>5 class). Regional differences in the density of potential integration sites for different transposable elements in the *D. melanogaster* genome have been reported by others. Smith et al. (1993) have examined the distribution of *hobo* integrations on chromosome 3 and compared it to similar data for *P*-elements (Fig. 7). Two general trends were observed. *P*-elements tended to be clustered in the proximal region of 3R, while the *hobo* elements tended to be found in the more distal locations of each arm. The pattern of *Hermes* integration did not show any regional tendencies and showed little overall similarity to the pattern of *hobo* elements (Fig. 7). *Hermes* had preferential integration

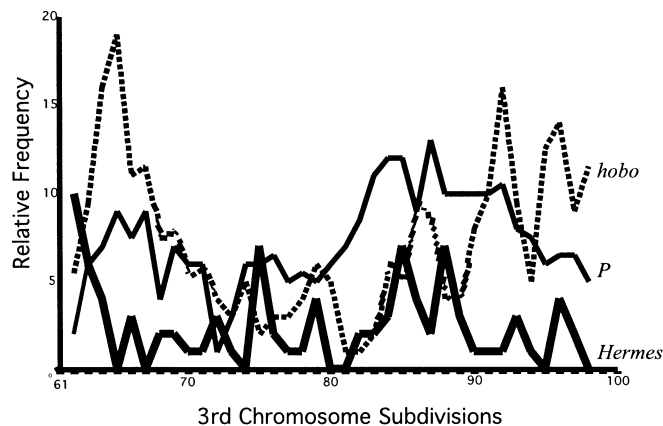


Fig. 7 Distribution of *Hermes*, *hobo*, and *P*-elements integration sites on chromosome 3. *P*-element and *hobo* data redrawn from Smith et al. (1993). The *y*-axis is calibrated in arbitrary units that are not comparable between elements

sites in both proximal and distal chromosomal positions. At the level of chromosomes and major chromosomal subdivisions, *Hermes* appears to differ distinctly in its mobility properties from the *P* and *hobo* elements. The difference between *hobo* and *Hermes* is particularly notable because of their high degree of relatedness and ability to interact (Sundararajan et al. 1999; Warren et al. 1994).

The analysis of integration events arising from single germ-line elements revealed evidence of local hopping. Local hopping refers to the tendency of an element to reintegrate preferentially at physically linked target sites. The phenomenon of local hopping was first described for *Ac* in maize, where 61% of the reinserted *Ac* elements remained close to the original site of integration (Greenblatt 1984). Numerous additional examples of local hopping of *Ac* in maize have been reported, and jump sizes have ranged from as few as 6 bp to several kb (Dowe et al. 1990; Peterson 1990; Grotewold et al. 1991; Chen et al. 1992; Weil et al. 1992). In *Arabidopsis*, *Ac* shows similar behavior, with 68% of elements transposing to linked sites (Bancroft and Dean 1993; Keller et al. 1993). *Ac* and *Ds* elements have not shown local hopping behavior in tomato, however (Yoder 1990; Osborne et al. 1991; Belzile and Yoder 1992). Similarly, *P*-elements from *D. melanogaster* also show local hopping behavior (Tower et al. 1993; Zhang and Spradling 1993; Golic 1994). The extent to which *P*-elements prefer linked to unlinked sites varies, and has been found to range from 20% to 80% (Zhang and Spradling 1993; Golic 1994). Newfeld and Takaesu (1999) reported data that suggested that the *hobo* element may also display local hopping behavior. These investigators reported the recovery of two integrations of *hobo* following transposition from a donor site approximately 25 kb away. We detected clear evidence of local hopping by *Hermes* in the soma of *D. melanogaster*. On average 39% of the *Hermes* transpositions recovered in this study that originated from a single donor element were

intrachromosomal, and 17% were within the same numbered division of the polytene chromosome. An examination of the positions of the re-integrated *Hermes* elements lying in the same numbered polytene chromosome division as the donor element revealed the scale at which local hopping can occur (Fig. 4). Within the collection of 25 intradivisional local hops recovered in this study jump size ranged from 1 bp to over 400 kb, with the median value being approximately 1 kb (1077 bp). In the five experiments included in this analysis all had at least one local jump (of approximately 1 kb or less) from the site of the donor element, and 10% of new insertions (16/149) were to sites within 2 kb of the donor. In addition, there appeared to be a tendency for local hops to accumulate on one side of the donor element (Fig. 4). These data support the growing body of evidence indicating that local hopping is a general characteristic of *hAT* elements, and may be a common characteristic of Class II transposable elements. While the mechanistic aspects of Class II element transposition are not yet sufficiently well known to allow us to understand the basis for local hopping, it may reflect an aspect of the transposition process that could be exploited in attempts to use elements such as *Hermes* as germ-line transformation vectors. Local hopping seems to imply that physical proximity of the donor to a target sequence increases the probability of the target being utilized. The association of the donor and target has been implicated in the movement of some plant transposable elements (Robbins et al. 1989). Use of elements like *Hermes* as insect transformation vectors currently involves microinjecting a mixture of plasmid DNA containing the donor element and the appropriate transposase gene into preblastoderm embryos as a means of introducing the donor element into the nuclei. The syncytial nature of young embryos makes this approach feasible, but clearly only a small fraction of the injected donor plasmids will be contained in nuclei following this process. Improvements in the frequency of transposition might be expected if the concentration of donor plasmid DNA in the nucleus could be increased and physical association of the donor plasmid and chromosome targets in the nucleus could also be promoted. Association of the plasmids and the chromosomes, perhaps by tethering with a multivalent DNA-binding protein, would make the chromosomes "local" to the donor element and increase the frequency of chromosomes being used as transposition targets.

Apart from what we have classified as local hopping, *Hermes* shows distinct regional preferences in its integration behavior. Certain regions of the genome, as defined by numbered divisions of the polytene chromosome, are preferred as integration sites, with these regions being targeted repeatedly by *Hermes*. These patterns cannot be explained by the non-uniform size of numbered chromosomal segments, and thus appear to be clusters. This clustering of independent transposition events within a region of the chromosome appears to reflect an undefined aspect of the transposition

process and how it might be influenced by the chromatin landscape. With one exception, none of the instances of clustering observed could be correlated with a common feature of the chromosome or the genes within the region concerned. The clustering observed in division 5, however, was unique, and reveals a property of *Hermes* that may also be common to Class II transposable elements. Eight of the 11 integration events that occurred in division 5 (3.2% of all remobilizations included in this study) were within the 2.7-kb segment of DNA upstream of the cytoplasmic actin gene, *actin5C*. What makes this particular cluster of *Hermes* integrations notable is the fact that the *Hermes* transposase ORF contained on *Hermes* QH7011 is regulated by the distal promoter of *actin5C*, contained on a 2.7-kb fragment derived from the plasmid pCaSperR-act (Thummel et al. 1988). All eight of the transposition events in the subdivision 5C occurred within the 2.7-kb region present on *Hermes* QH7011. The strong clustering of transpositions in a sequence that is homologous to a sequence contained on the vector has been referred to as homing. Homing was first described for *P*-elements that carried specific regulatory sequences from the *Drosophila engrailed* gene and from the homeotic complexes *Bithorax* and *Antennapedia* (Hama et al. 1990; Engstrom et al. 1992; Hudson et al. 1995; Bender and Hudson 2000). Hama et al. (1990) found that seven out of 20 primary germ-line integration events that arose from the injection of a *P*-element with a 3.4-kb fragment containing the regulatory region of the *engrailed* gene occurred in the vicinity of the *engrailed* gene. Similarly, Kassis et al. (1992) reported strong regional specificity of *P*-element insertion that correlated with the sequences contained on the element. In this case, again, the sequences on the donor element and the target were regulatory sequences from the *engrailed* gene. These initial reports of homing and preferential insertion all involved elements with binding sites for *Polycomb*-group proteins (*Polycomb*-group Response Elements, PREs). Furthermore, the degree of precision of the homing phenomenon was low, and resulted in regional clustering of insertions within a 200-kb region, with no evidence of strong sequence specificity. More recently, Taillebourg and Dura (1999) reported transposable element homing of *P*-elements with 11-kb and 1.6-kb fragments of genomic DNA containing the 5' region of the *linotte* gene (*lio*). Up to 20% of remobilized elements containing *lio* regulatory sequences inserted into the resident *lio* gene. Insertions in this case were highly precise and most occurred within a 36-bp fragment. The homing in this case was independent of PREs. *Hermes* homing in this study was very similar to that observed by Taillebourg and Dura (1999). It was highly precise and appeared to be sequence dependent, with integrations only occurring within the 2.7-kb distal promoter region of *actin5C*. These data confirm and extend the conclusions of Taillebourg and Dura (1999) that homing is a PRE-independent phenomenon. Furthermore, these data

indicate that homing is not an element-specific phenomenon, but may be a general characteristic of Class II elements. We suggest that homing is a special case of local hopping. As discussed above, a number of Class II elements display a bias in their integration-site preferences that is determined, in part, by physical proximity between donor elements and target sites. The presence of transgene regulatory sequences on Class II transposable elements may promote the physical association of donor elements and target sites, perhaps by tethering them with proteins binding to common DNA binding sites (Kassis et al. 1992; Taillebourg and Dura 1999). Local hopping of tethered elements will result in a distribution of integrations that is consistent with what has been described as homing. Tethering has been shown to serve as a mechanism for manipulating the distribution of retrovirus integrations. Bushman (1994) showed that adding an exogenous DNA binding domain (lambda phage repressor protein) to human immunodeficiency virus 1 integrase biased the integration pattern of HIV to regions near and around lambda phage operator sequences. In this case, tethering of the recombinase was sufficient to bias integration patterns. While tethering donor elements and target sites seems feasible when the donor elements are contained on plasmids, it may be problematic when donor elements are themselves integrated at other and distant regions of the genome. If, however, excised elements exist as at least semi-stable intermediates, then tethering may be an influential aspect of target site selection *in vivo*.

*hAT* elements create 8-bp duplications upon integration, probably through the repair of 8-bp single stranded regions generated by the staggered nicking of target DNA during integration. The duplicated sequence has commonly been referred to as the target site, and it, together with approximately 15 bp of flanking DNA on both sides of the element, plays an important role in determining the target properties of a sequence (Saville et al. 1999). Saville et al. (1999) found that plasmid DNA sequences that were integration hot spots for *hobo* could be moved around on a fragment of DNA approximately 40 bp in length without losing the targeting properties of the sequence. These investigators found that the primary nucleotide sequence of the actual integration site was playing some role in the targeting process. The information in a *hobo* target site was qualitatively and quantitatively similar to that found in *Hermes* target sites. These authors also concluded that the primary nucleotide sequence of the flanking DNA was playing little role in influencing the targeting properties, and that structural features were likely to play a more important part, as has recently been suggested for *P*-element insertion sites (Liao et al. 2000). The analysis of *hobo* and *Hermes* target sites has been based on the integration of plasmid-borne transposable elements into a plasmid target sequence. Analysis of *hobo* and *Hermes*

target sites based on chromosomal integration events has so far been quite limited. However, the analysis reported here also reveals that the target site contains little information in its primary sequence (Fig. 6). Only positions 2 and 7 contained notable amounts of sequence information and, as was found with plasmid-based assays, T and A, respectively; were the most common nucleotides at these positions. Target-site selection, therefore, follows rules that are independent of whether the target sequence is chromosomal or extrachromosomal.

*Hermes*, together with other *hAT* elements, is proving to have the potential to be developed into a powerful genetic tool. Our analysis of its mobility properties in the soma of a foreign host is beginning to reveal characteristics of *Hermes* that show it to be similar, in some ways, to transposable elements such as *P* that are used in well established and powerful gene finding and analysis systems. These common properties, such as local hopping and homing, probably reflect general characteristics of Class II transposable elements that are dependent in some way on their common mechanism of transposition. This analysis was confined to *Hermes* elements moving in somatic tissue, and our working hypothesis is that local hopping and homing are characteristics of germ-line transposition of *Hermes* elements as well. Currently no data exist that would cause us to reject that hypothesis. This analysis also revealed distinctions between the behavior of *Hermes*, *P* and *hobo*, indicating that the diversity of transposable element-based genomics tools currently being developed will not only expand the number of insect species for which germ-line transformation will be possible, but will provide us with tools with distinct capabilities within a species. Understanding the behavior of the genetic tools used in the creation of transgenic organisms will permit us to maximize their utility and minimize the risks associated with their use in insects of economic and health significance. Indeed the question of risk associated with the use of these elements in new, non-host species, has not been addressed at any significant level. The mobility properties, i.e., the rate of movement, the type of movement and factors affecting movement, of elements like *Hermes* when placed in new genomes will directly affect the success of population replacement programs in which these elements are applied as genetic drive agents. Furthermore, measurement of these properties will enable meaningful evaluations to be made of the likelihood of cross-species mobility. Such estimates may well prove to be essential requirements in order for release of transgenic insects, no matter how potentially beneficial, to be permitted.

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