hobo Enhancer Trapping Mutagenesis in Drosophila Reveals an Insertion Specificity Different from *P* Elements

Desmond Smith,* Jay Wohlgemuth,*[1] Brian R. Calvi,* Ian Franklin[†2] and William M. Gelbart*[3]

**Department of Cellular and Developmental Biology, The Biological Laboratories, and †Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138*

Manuscript received March 31, 1993
Accepted for publication August 11, 1993

ABSTRACT

P element enhancer trapping has become an indispensable tool in the analysis of the *Drosophila melanogaster* genome. However, there is great variation in the mutability of loci by these elements such that some loci are relatively refractory to insertion. We have developed the *hobo* transposable element for use in enhancer trapping and we describe the results of a *hobo* enhancer trap screen. In addition, we present evidence that a *hobo* enhancer trap element has a pattern of insertion into the genome that is different from the distribution of *P* elements in the available database. Hence, *hobo* insertion may facilitate access to genes resistant to *P* element insertion.

THE *hobo* and *P* transposable elements are both members of a family of transposons with terminal inverted repeats which probably transpose via DNA intermediates [for reviews of *P* elements see Engels (1989) and Rio (1990); for reviews of *hobo* elements see BLACKMAN and GELBART (1989) and Gelbart and Blackman (1989)]. However, despite the similarities between the elements, there are also some differences between them. There is little obvious similarity between the 8-bp host genomic sequences duplicated upon insertion of *hobo* and *P* elements, with the possible exception of the last two nucleotides, which may show a preference for the sequence AC (STRECK, MACGAFFEY and BECKENDORF 1986). In addition, there is no homology between the *P* transposase and the putative *hobo* transposase (CALVI *et al.* 1991). For prokaryotic inverted repeat transposons it is well established that the structure of the transposase affects the DNA sequences in which the transposon inserts (PLASTERK 1990).

The advent of single *P* element insertional mutagenesis has revolutionized Drosophila molecular genetics (COOLEY, BERG and SPRADLING 1988; COOLEY, KELLEY and SPRADLING 1988; BERG and SPRADLING 1991). In this technique, just one marked *P* element is used to mutagenize the genome and the subsequent molecular analysis of the resulting mutants is thereby greatly simplified in comparison to previous approaches using P-M hybrid dysgenesis in which multiple unmarked elements were mobilized. This approach has been usefully extended by the enhancer trap technique in which a reporter gene driven by a weak promoter is placed within the *P* element (O'KANE and GEHRING 1987; BELLEN *et al.* 1989; BIER *et al.* 1989; WILSON *et al.* 1989). The weak promoter is susceptible to the influence of adjacent genomic enhancers and the resulting pattern of reporter gene expression may reveal the pattern of expression of the gene into which the *P* element has landed. Tens of thousands of single *P* element enhancer trap lines have been generated (O'KANE and GEHRING 1987; BELLEN *et al.* 1989; BIER *et al.* 1989; WILSON *et al.* 1989). However, it is unlikely that a complete sampling of the genome will be achieved using *P* elements alone, since genes display a wide variation in their receptivity to insertion (KIDWELL 1986; ENGELS 1989). Some genes are hot spots for *P* element insertion, whereas others are cold spots and are therefore relatively refractory to insertion. One example of an apparent cold spot is the ADH gene, for which 10 million flies from a P-M hybrid dysgenic cross were screened without successfully obtaining a *P* element insertion (KIDWELL 1987). It has been estimated that roughly 50% of Drosophila genes are mutable by P-M hybrid dysgenesis (KIDWELL 1986) and it is thought that a similar proportion of genes is mutable using single *P* element mutagenesis (COOLEY, KELLEY and SPRADLING 1988). These observations suggest that many genes will not be readily identified using *P* element mutagenesis alone.

A more complete distribution of insertions throughout the genome could be achieved with *hobo* insertional mutagenesis, if indeed *hobo* and *P* elements tend to insert into different subsets of loci. For this purpose, we have developed and exploited a *hobo* enhancer trap. We performed a *hobo* enhancer trap mutagenesis

[1] Present address: Stanford University School of Medicine, 851 Welch Road, Palo Alto, California 94304.
[2] Present address: CSIRO, Division of Animal Production, P.O. Box 239, Blacktown, New South Wales 2148, Australia.
[3] To whom correspondence should be addressed.

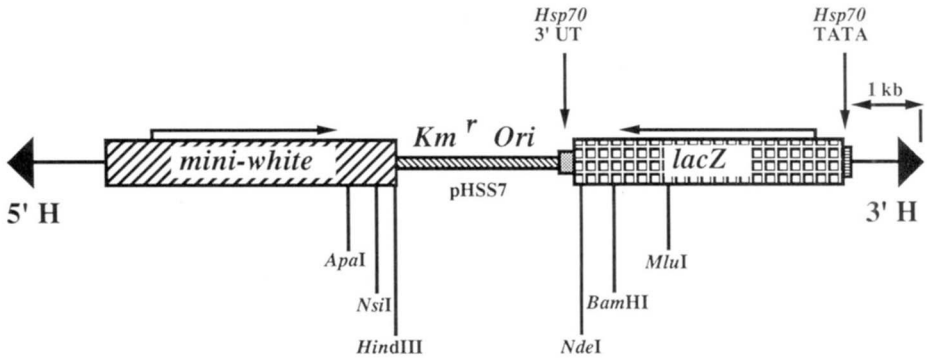


FIGURE 1.—The *hobo* enhancer trap *H[pHLw2]*. *Km*^{r}, *kanamycin* resistance; *Ori*, plasmid origin of replication; *lacZ*, β-galactosidase gene; 3′ UT, 3′-untranslated region. Restriction enzyme sites useful for plasmid rescue are shown.

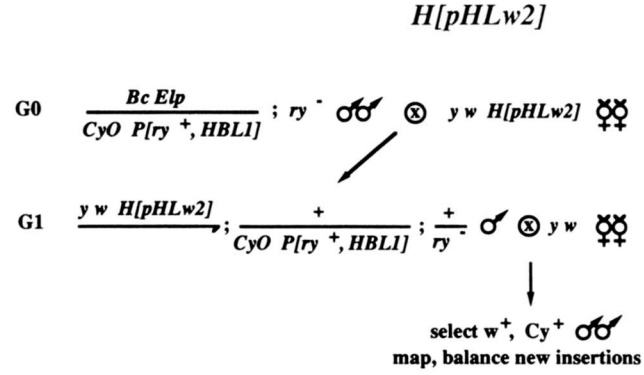


FIGURE 2.—The genetic scheme by which the *hobo* enhancer trap was mobilized from the *X* chromosome to the autosomes.

and compared the pattern of insertion of the *hobo* enhancer trap into the genome with that of reported *P* elements in order to address whether the two elements preferentially insert into different regions.

MATERIALS AND METHODS

Construction of pHLw2: Molecular biology techniques were performed as previously described (SAMBROOK, FRITSCH and MANIATIS 1989). The *Eco*RI site at nucleotide 1159 of pHFL1 (CALVI *et al.* 1991) was disabled by digestion with *Eco*RI and filling in with Klenow. The resulting plasmid was digested with *Eco*RI, the ends filled in using Klenow and the DNA digested with *Hin*dIII. The resulting 6.2-kb fragment was ligated to the 4.8-kb *Kpn*I-*Hin*dIII fragment from pHZ50PL (HIROMI and GEHRING 1987), the *Kpn*I terminus having been polished using T4 DNA polymerase. The 6.5-kb *Bsu*36I-*Xba*I fragment from this plasmid was ligated to the 5.9-kb *Bsu*36I-*Xba*I fragment from pFZ (MLODZIK and HIROMI 1992). The resulting plasmid was digested with *Xba*I and ligated to the 4.2-kb *Spe*I fragment from pCahsneo-miniwhite (V. PIRROTTA, personal communication) thus generating pHLw2. This plasmid contains 0.3 kb of upstream sequences from a *Hsp70* gene at cytological band 87C1 linked upstream of the *lacZ* gene. The *lacZ* gene is driven by the *Hsp70* TATA box which acts as a weak promoter but lacks the heat shock-inducible sequences. Trailer sequences (0.9 kb) from a *Hsp70* gene at cytological band 87A7 are attached downstream of the *lacZ* gene. pHLw2 also contains the *mini-white* gene (PIROTTA, STELLER and BOZZETTI 1985) and pHSS7 (SIEFFERT *et al.* 1986) which can be used for plasmid rescue (PIROTTA 1986). Another plasmid, pHLr1, consisted of the *lacZ* gene fused in frame to the putative *hobo* transposase (CALVI *et al.* 1991), such that the *hobo* promoter would act as an enhancer trap, but the construct did not appear to be effective in this regard (D. SMITH, B. R. CALVI and W. M. GELBART, unpublished data).

TABLE 1

Jump rates of *hobo* insertions in the *X* chromosome

Donor chromosome	Cytological location of *hobo*	No. of G_1 crosses[a]	No. of jumps[b]	Jump rate[c] (%)
A	3E1,2	157	6	4
B	7D14-17	59	3	5
C	17D	204	20	10
D	20C	49	2	4
E	ND[d]	50	0	0
A + C	3E1,2 + 17D	195	32	16

[a] Number of fertile crosses.
[b] Jumps were defined following COOLEY, BERG and SPRADLING (1988) as the number of G_1 vials containing at least one fertile w^+ son that does not contain *P[ry*$^+$*, HBL1]*.
[c] Jump rate was defined following COOLEY, BERG and SPRADLING (1988) as the percent of jumps/fertile G_1 cross.
[d] ND = not determined.

Genetic strains: All mutations are described in LINDSLEY and ZIMM (1992). Unless otherwise indicated all strains are E, that is, devoid of *hobo* elements (STRECK, MACGAFFEY and BECKENDORF 1986; B. R. CALVI and W. M. GELBART, unpublished data).

Production of transgenic flies: *y* w^{67c23} embryos were injected as described (SPRADLING 1986) with pHLw2 DNA (0.6 mg ml^{-1}) together with pHBL1 (0.15 mg ml^{-1}) helper DNA (CALVI *et al.* 1991). G_0 flies were mated singly and transgenic flies selected as w^+ G_1 progeny. Insertions were mapped and balanced using the strains *y* w^{67c23}; *Gla/SM6a* and *y* w^{67c23}; D^3 *gl/TM3, Sb Ser*.

Transposition crosses: A stable source of *hobo* transposase, *P[ry*$^+$*, HBL1]*, on the *CyO* chromosome (CALVI *et al.* 1991; B. R. CALVI and W. M. GELBART, unpublished data) was used to jump the *hobo* enhancer trap from the *X* chromosome to the autosomes. Jump rates were calculated following COOLEY, BERG and SPRADLING (1988) as the proportion of fertile cultures that produced at least one fertile w^+, Cy^+ son. New autosomal insertions were mapped and bal-

TABLE 2

Mode of production of the *hobo* enhancer trap lines

Donor element	No. of lines	No. of lines mapping to chromosomes *X*, *2* or *3* analyzed by *in situ* hybridization
Injection[a]	30 (including 2 on *X* chromosome)	16 (including 2 on *X* chromosome)
A	195	59
C	234	68
A and C	104	12
Miscellaneous[b]	49 (including 6 on *X* chromosome)	7 (including 3 on *X* chromosome)
Total lines	612 (including 8 on *X* chromosome)	162 (including 5 on *X* chromosome)

[a] Approximately 1,000 embryos were injected and 320 fertile G_0 adults were obtained from these embryos.
[b] These lines were produced as a result of mobilization of *hobo* insertions from the autosomes. These mobilizations were performed in order to obtain further *X* insertions for transposition to the autosomes as described in RESULTS.

anced using the strains *y* w^{67c23}; *Gla/SM6a* and *y* w^{67c23}; D^3 *gl/TM3, Sb Ser*.

Synthesis of a recombinant *X* chromosome containing two *hobo* enhancer trap insertions: In order to increase the jump rate, a recombinant *X* chromosome containing two insertions of the *hobo* enhancer trap (insertions A and C, see RESULTS) was synthesized using the fact that the *mini-white* gene confers a less than wild type level of w^+ eye color upon the flies (PIROTTA, STELLER and BOZZETTI 1985). This means that flies with two copies of a transposon bearing the *mini-white* gene possess a darker w^+ eye color than flies bearing one copy. The fact that the recombinant chromosome contains two transposons was confirmed by *in situ* hybridization.

Embryonic β-galactosidase staining: Embryos were stained for β-galactosidase activity (BELLEN *et al.* 1989; BIER *et al.* 1989) and with anti-β-galactosidase antibodies as described (BIER *et al.* 1989).

***In situ* hybridization:** Chromosomal *in situ* hybridization was performed as described (ENGELS *et al.* 1986) using pHLw2. In addition to the *hobo* enhancer trap, this probe hybridized positively to the *white* gene at 3C2 and with variable strength to the *Hsp70* genes at 87A7 and 87C1. For homozygous lethal *hobo* insertions on the second chromosome, *y* w^{67c23};*H[pHLw2]/SM6a* males were mated with *y* w^{67c23};*Bc Elp/CyO* virgin females. Cy^+ males resulting from this cross were mated with *y* w^{67c23} virgin females and Bc^+ larvae resulting from this cross were used for chromosomal *in situ* hybridization. For homozygous lethal *hobo* insertions on the third chromosome, *y* w^{67c23};*H[pHLw2]/TM3, Sb Ser* males were mated with *ru h th st cu sr* e^s *Pr ca/TM6B, Tb* virgin females. Sb^+, Pr^+ males resulting from this cross were mated with *y* w^{67c23} virgin females and Tb^+ larvae resulting from this cross were used for chromosomal *in situ* hybridization.

RESULTS

Jump rates of *H[pHLw2]*: Five separate *X*-linked insertions of the *hobo* enhancer trap *H[pHLw2]* (Figure 1) were assayed for their ability to transpose to the autosomes using the stable source of *hobo* transposase *P[ry*$^+$*, HBL1]* (CALVI *et al.* 1991; B. R. CALVI and W. M. GELBART, unpublished data). The genetic crosses employed are shown in Figure 2, and the jump rates of the lines are shown in Table 1. Line A was derived from injection. Lines B to E were derived by jumping *hobo* enhancer traps on chromosome *3* resulting from the injection experiment onto the *X* chromosome. In order to increase the jump rate, a recombinant *X* chromosome containing both insertions A and C was synthesized and the jump rate of this chromosome was approximately equal to the combined jump rate of the chromosomes containing the single insertions. Most of the lines obtained in the screen were created using insertions A, C, or the A, C recombinant *X* chromosome (Table 2).

Embryonic staining patterns: A total of 612 lines were established and assayed for embryonic β-galactosidase expression. Eighty percent of the lines displayed staining. However, many of these staining patterns were either uniform (7%) or were repeatedly observed patterns (46%). The two repeatedly observed patterns were staining of the dorsal longitudinal trunks of the tracheae from stage 15 onward and staining of the proventriculus from stage 16 onward. These repeatedly observed patterns were presumed to represent an intrinsic property of the enhancer construct itself. Such a phenomenon has been observed with a *P* element enhancer trap (BELLEN *et al.* 1989) although the repeatedly observed pattern was different. Approximately 30% (181/612) of the *hobo* lines were judged to display specific staining. In contrast, two *P* element enhancer traps showed specific staining in 65% of lines (BELLEN *et al.* 1989; BIER *et al.* 1989). The lower frequency of specific staining observed for the *hobo* enhancer trap may be due to the relatively large distance (1.0 kb) from the weak promoter to the 3′ terminus of the element. By comparison, the *P* element enhancer traps employed the *P* element's weak promoter which is adjacent to the 5′ terminus of the element. *P* element enhancer traps with weak promoters situated some distance internal to the termini of the element show lower frequencies of specific staining than those which employ the *P* element promoter (PERRIMON *et al.* 1991). A wide variety of different staining patterns was observed (Figures 3-6) using the *hobo* enhancer trap. Of the specifically staining lines, most showed staining either

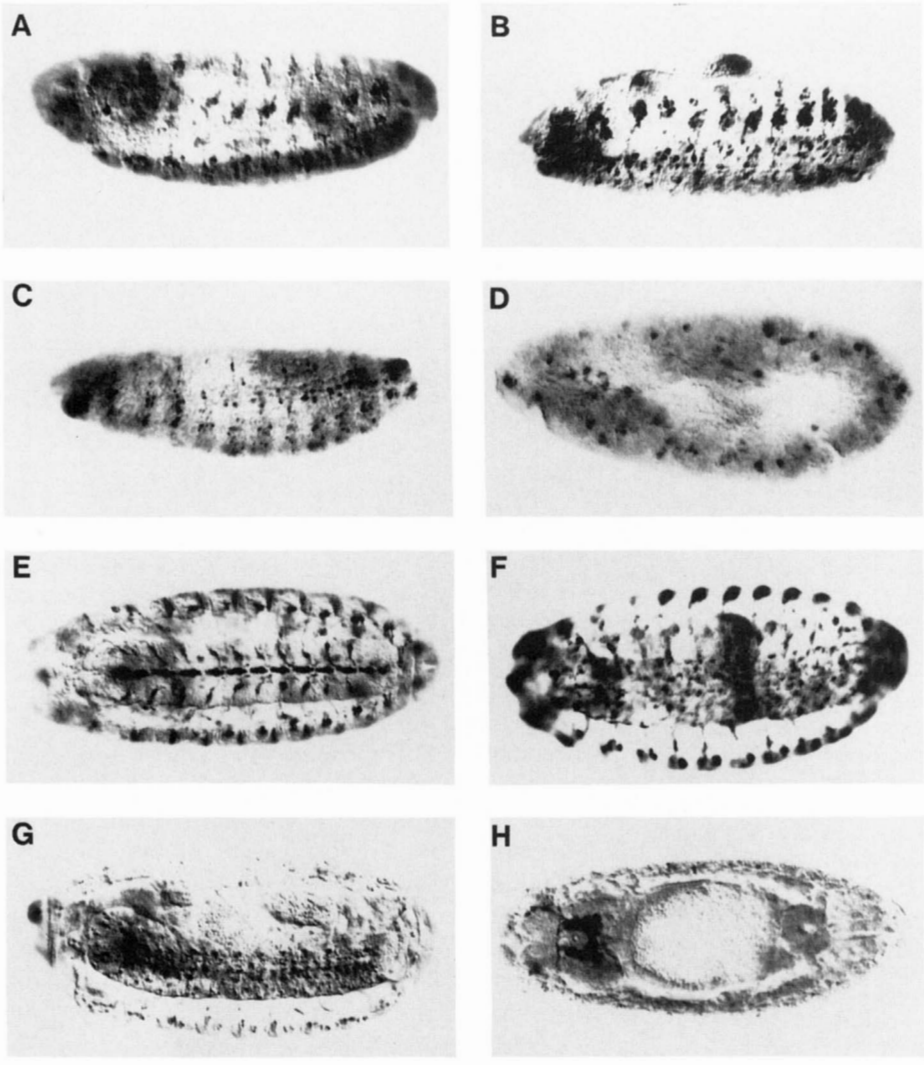


FIGURE 3.—*lacZ* expression patterns of lines displaying staining in the central nervous system (CNS) and peripheral nervous system (PNS), as revealed by anti-β-galactosidase antibodies. Unless otherwise stated, all embryos are shown anterior to the left and dorsal up. When known, the cytological location of the enhancer trap insertion is shown. (A) Line 158 (96E): staining of the dorsal, lateral and ventral clusters of the PNS (B) Line 422 (chromosome *3*): staining of the lateral and ventral clusters of the PNS. (C) Line 244 (30F): staining of cells in the PNS and CNS, together with additional cells. (D) Line 470 (30A7,8): staining of a subset of neuroblasts and PNS precursor cells. (E) Line 250 (63E): staining of midline cells in the ventral nerve cord (VNC) (ventral view). (F) Line 422: staining of cells in the VNC and the midgut (ventral view). (G) Line 492 (66F1,2): staining of cells in the VNC (ventral view). (H) Line 414 (chromosome *3*): staining of the supraesophageal commissure (dorsal view).

in the nervous system (87/181 = 48%) or in the gut (66/181 = 37%). Fewer showed staining in mesodermal derivatives (12/181 = 7%). This profile of staining frequencies is very similar to that observed with *P* elements. The number of lines showing staining in various tissues is shown in Table 3. The β-galactosidase gene in the *hobo* enhancer trap had no nuclear localization signal and consequently staining was observed both in the nucleus and cytoplasm.

Pattern of insertion of the *hobo* enhancer trap into the genome: The positions of *hobo* enhancer trap insertions were determined by chromosomal *in situ* hybridization for 157 lines mapping to the second or third chromosomes (Table 2 and Figure 7). Apart from the exceptions described below, 93% (132/142) of the *hobo* enhancer trap lines proved to contain a single element.

There appeared to be no obvious differences between the patterns of insertions with regard to the origin of the donor element. This is similar to findings described for *P* elements (BERG and SPRADLING 1991). However, a disproportionately large number of *hobo* insertions were found at 55A when insertion A was the donor element. Of the 21 second chromosome lines derived from insertion A that were also analyzed by *in situ* hybridization, 15 had in common a *hobo* insertion at 55A. These insertions were molecularly identical as judged by Southern blotting (data not shown). In addition, Southern blot analysis and *in situ* hybridization showed that these insertions were nearly always associated with another element elsewhere on 2R (14/15). In 9/15 of the lines the companion *hobo* insertions were resolvable from the element at 55A by *in situ* hybridization. The 55A-associated *hobo* insertions are shown in Figure 8. These observations are best explained by the phenomenon of local jumping such as has been described for *P* elements (TOWER *et al.* 1993; ZHANG and SPRADLING 1993) and which has been suggested to occur for *hobo* elements (SHEEN, LIM and SIMMONS 1993). It is particularly tempting to speculate that local jumping may occur for the *hobo* element, because of its relationship to the *Ac/Ds* transposable element of *Zea mays* (CALVI *et al.* 1991). Local jumping was first described for the *Ac/Ds* element

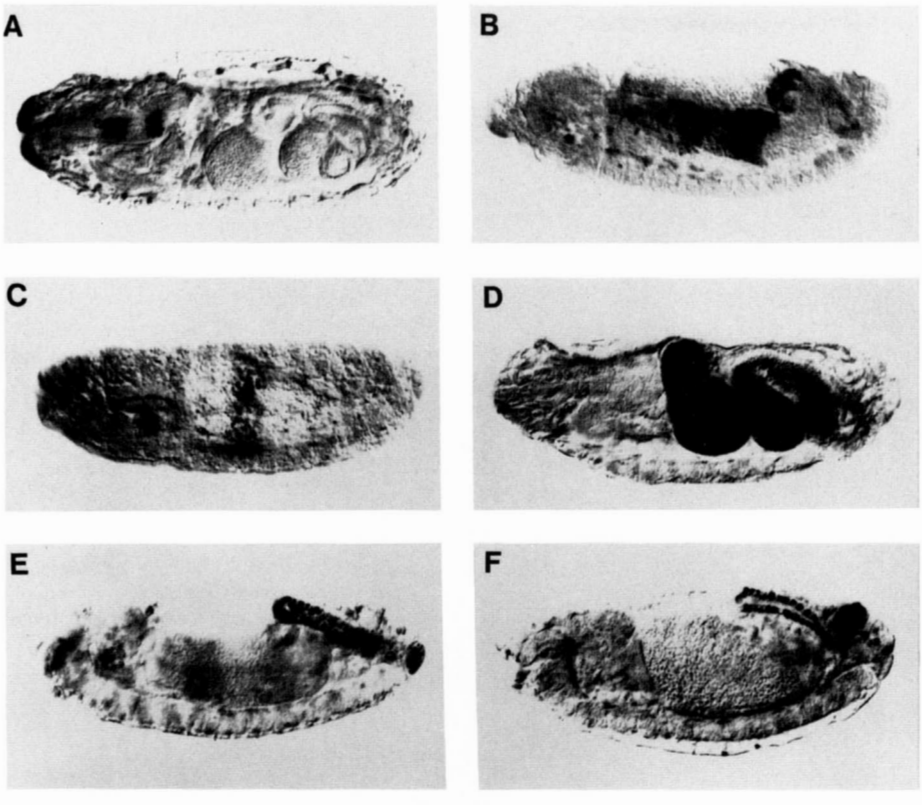


FIGURE 4.—*lacZ* expression patterns of lines displaying staining in the gut. (A) Line 47 (98A11,12): staining of the pharynx. (B) Line 423 (86C1,2): staining of the midgut and proximal hindgut (stage 13 embryo). (C) Line 98 (chromosome two): staining of the midgut at the nascent second constriction. (D) Line 393 (62B1,2): staining of the midgut. (E) Line 470 (30A7,8): staining of the hindgut. (F) Line 79 (62A): staining of a loop of cells in the hindgut which starts at one posterior spiracle and ends at the other. There is also staining of the posterior spiracles.

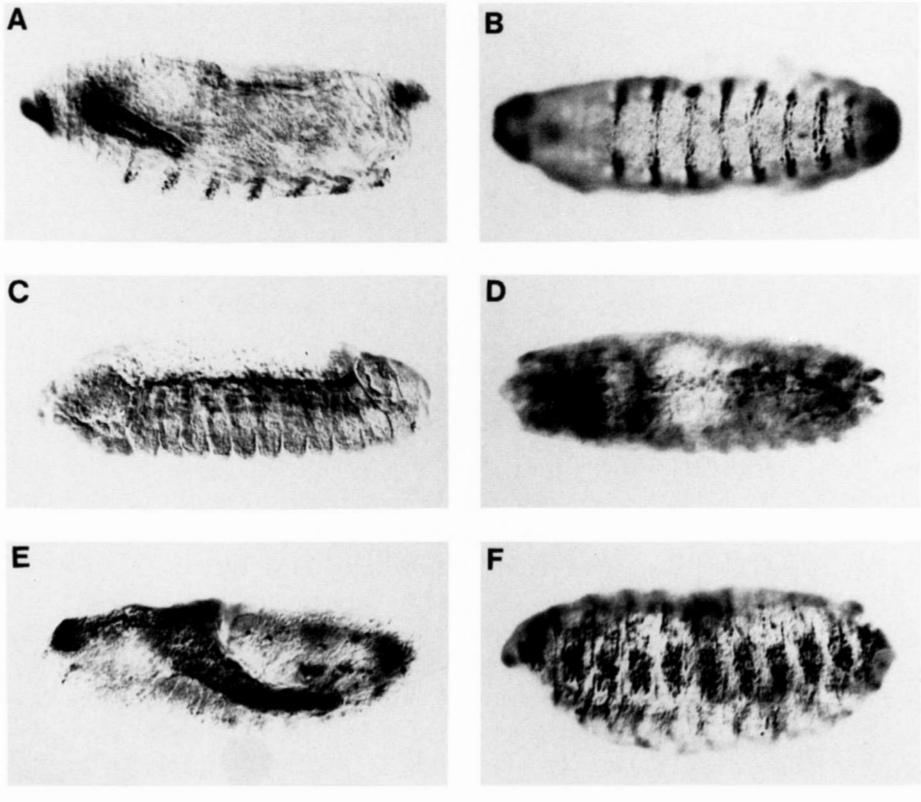


FIGURE 5.—Miscellaneous β-galactosidase expression patterns. (A) Line 12 (100C4,5): staining of the salivary glands and eight ventral epithelial abdominal stripes. (B) Line 12: ventral view showing the abdominal epithelial stripes. (C) Line 410 (59F3): staining of the cardial cells (dorsal-most staining row of cells) and the pericardial cells (more ventral cells) in a stage 13 embryo. Both of these cell types will contribute to the dorsal vessel. (D) Line 495 (chromosome 3): staining of the dorsal vessel (dorsal view). (E) Line 8 (65A): staining of the amnioserosa (stage 11 embryo). (F) Line 8: staining of a subset of muscle cells (stage 16 embryo).

(VAN SCHAIK and BRINK 1959) and has been well documented (see for example ATHMA, GROTEWOLD and PETERSON 1992; MORENO *et al.* 1992). Specifically, we suggest that if there was a phenotypically silent (w^-) *hobo* element at 55A which was segregating undetected in the stock bearing insertion A, local jumps from this element would give rise to a substantial fraction of phenotypically w^+ lines mapping to the second chromosome. The distribution of putative local jumps shown in Figure 8 suggests that this ap-

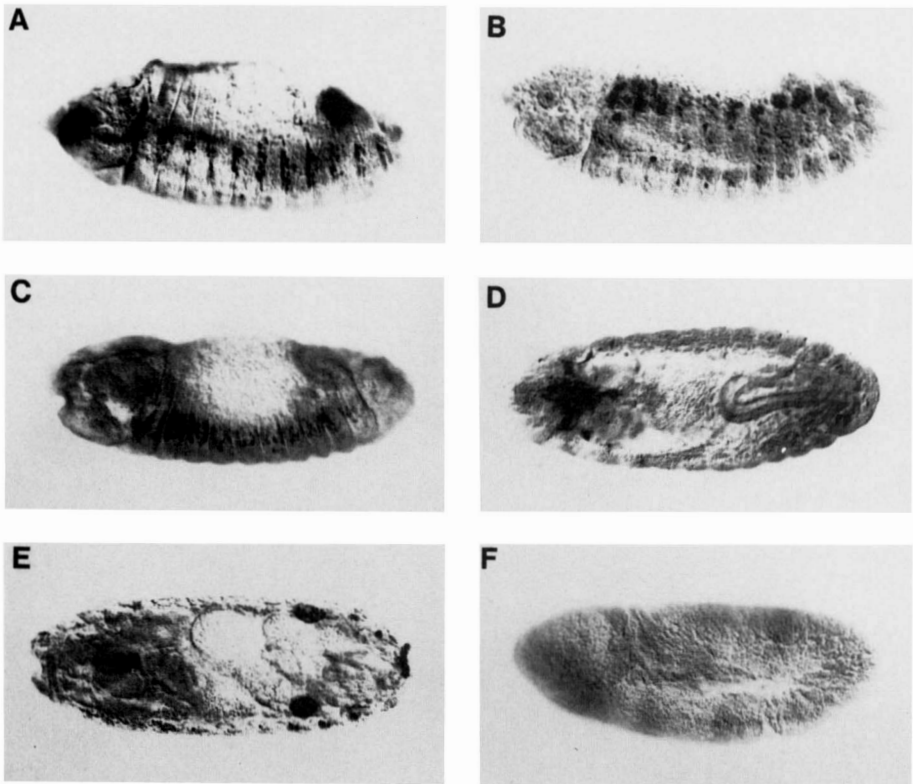


FIGURE 6.—Further β-galactosidase expression patterns. (A) Line 282 (55C): staining of the epidermis at the intersegmental furrrows and staining of the proximal part of the hindgut. (B) Line 153 (99A): staining of the dorsal part of the epidermis at the boundary with the amnioserosa (stage 14 embryo). (C) Line 510 (69D1–3): staining of the epidermis at the intersegmental furrows. (D) Line 146 (79D1,2): staining of the ring gland (dorsal view). (E) Line 65 (94A): staining of the gonads and the pharynx (dorsal view). (F) Line 33 (89A): staining of seven stripes of cells in an extended germ band embryo similar to a pair rule pattern. This pattern is first seen in the blastoderm and after germ band extension is completed evolves into a 14 stripe expression pattern reminiscent of the segment polarity genes.

TABLE 3

Profile of specific staining frequencies for the *hobo* enhancer trap

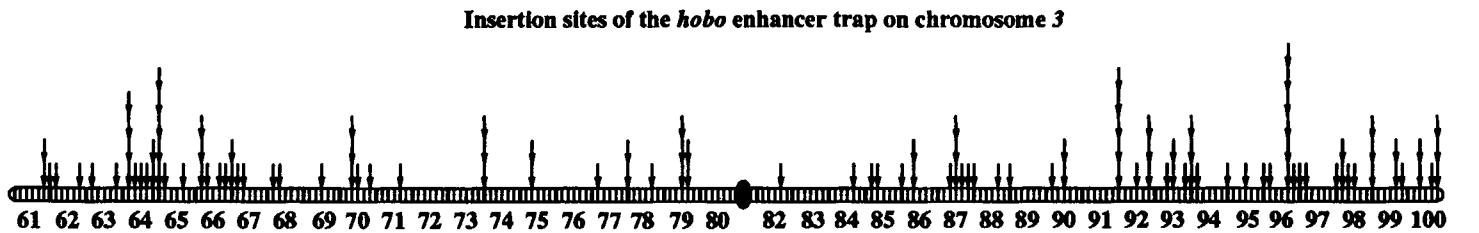
Tissue displaying staining[a]	No. of lines
CNS[b]	63
PNS[c]	70
Ring gland	4
Epidermis	20
Pharynx	7
Esophagus	5
Salivary glands	18
Midgut	52
Hindgut	15
Malpighian tubules	6
Anal plate	3
Muscle	6
Visceral mesoderm	10
Heart	7
Amnioserosa	6
Oenocytes	4
Posterior spiracles	14
Gonads	2

[a] Lines may be included in more than one category of specifically staining tissue.
[b] CNS = central nervous system.
[c] PNS = peripheral nervous system.

proach might be useful for mutagenesis of a conveniently sized segment of the genome.

How accurately does the enhancer trap pattern reflect the pattern of expression of nearby genes? For *P* element enhancer traps there have been a number of cases where it has been shown that the pattern of expression of the enhancer trap closely mimics the pattern of the gene next to which the transposon has inserted (BIER *et al.* 1989; WILSON *et al.* 1989; WILSON, BELLEN and GEHRING 1990). The same phenomenon appears to be true for the *hobo* enhancer trap. In one example, line 8, staining is first seen at stage 11, in the amnioserosa and the visceral and somatic mesoderm. In later stages after germ band retraction is completed, staining persists in the visceral mesoderm and a subset of muscles and in addition is observed in the epidermis in two to three cells at the anterior part of each segment adjacent to the intersegmental groove. Some aspects of this staining pattern are shown in Figure 3, E and F. The *hobo* insertion in this line is situated at 65A. The laminin A gene is known to be in this region, at 65A10–11 (MONTELL and GOODMAN 1988) and is a candidate for the locus identified by this enhancer trap. The pattern of expression of the laminin A gene (MONTELL and GOODMAN 1989) is very similar to the *hobo* enhancer trap. The gene is first strongly expressed in the mesoderm in extended germ band embryos. The gene then continues to be most strongly expressed in the somatic mesoderm which forms the body wall muscles and the visceral mesoderm, and in addition is expressed in the epidermis.

Another example is provided by line 79 whose insertion is located at 62A. The first staining is weakly displayed in one midline mesectodermal cell per seg-



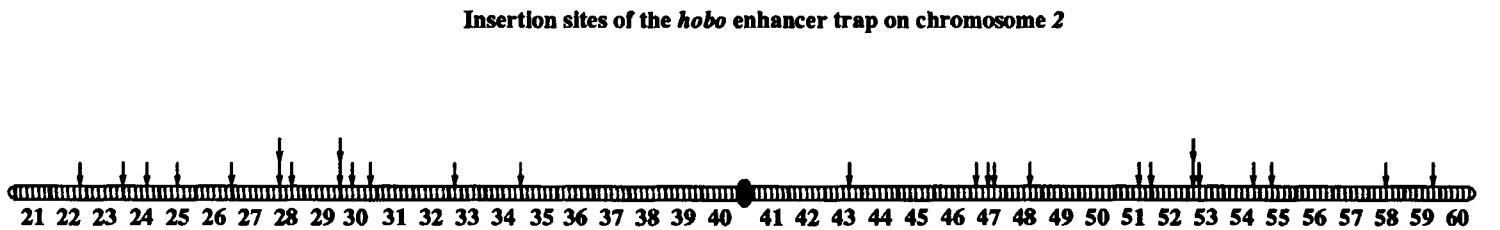


FIGURE 7.—Distribution of *hobo* enhancer trap insertions along the second and third chromosomes as determined by *in situ* hybridization.

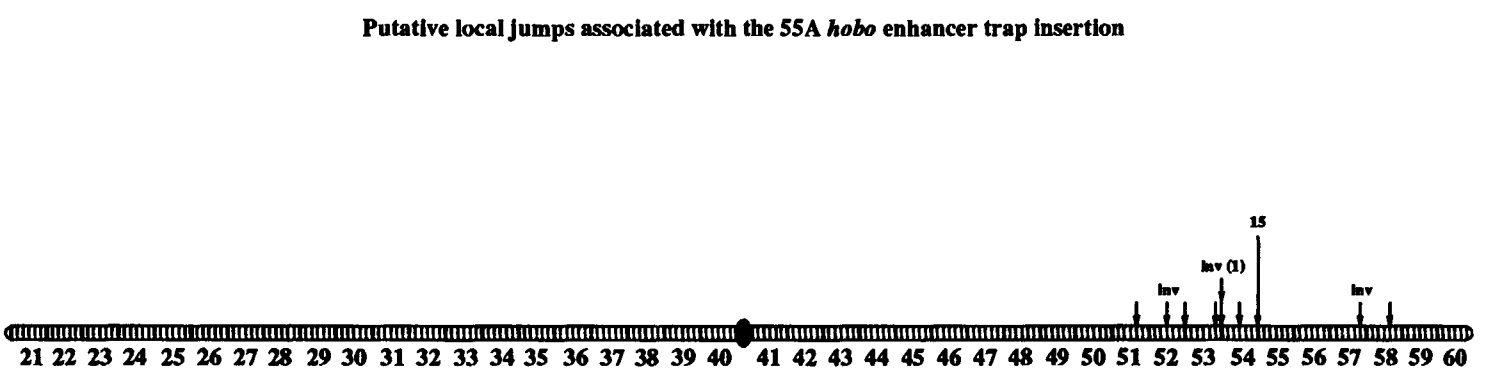


FIGURE 8.—Distribution of putative *hobo* enhancer trap local jumps associated with 55A as shown by *in situ* hybridization. Insertions marked Inv were associated with inversions between the putative local jump and the *hobo* insertion at 55A.

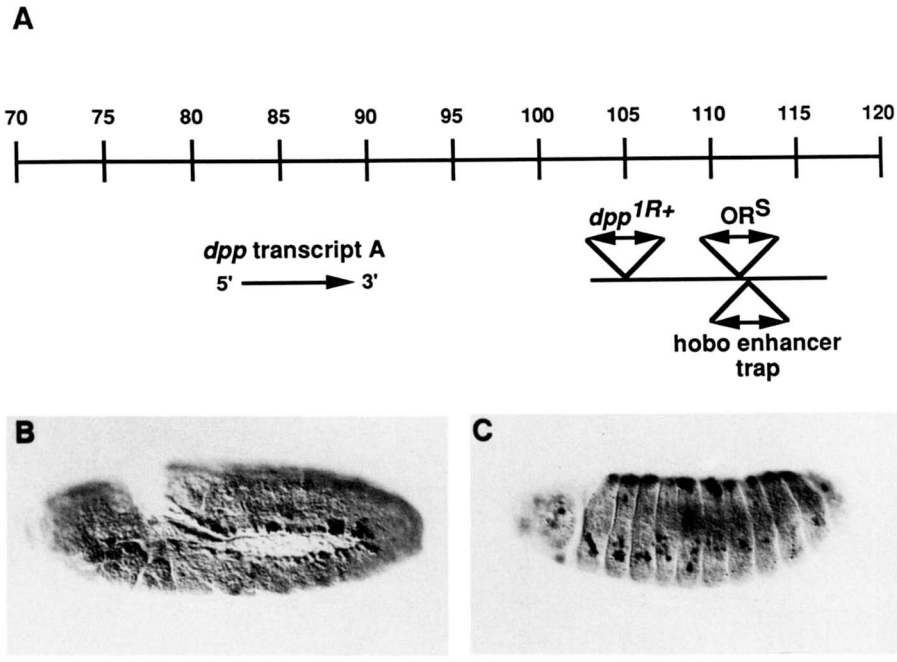


FIGURE 9.—A line containing a *hobo* enhancer trap inserted in the *disk* region of the *dpp* gene recapitulates part of the expression pattern of the gene. (A) The structure of the *dpp* gene. A detailed description of the gene can be found in ST. JOHNSTON *et al.* (1990). The co-ordinates of the gene (kb) and the extent of a major transcript are shown. The *disk* region is a large 3′-*cis*-regulatory region. The positions of two previously mapped independent *hobo* insertions into the *dpp* gene are shown, as is the position of the *hobo* enhancer trap of line 151. The direction of transcription of the *lacZ* gene in the *hobo* enhancer trap is from left to right. (B) Staining displayed by line 151 at the beginning of germ band retraction. (C) Staining at stage 14. Staining can be seen to occur in the dorsal epidermis, the cephalic and thoracic imaginal disk primordia and the peripheral nervous system.

ment in the germ band extended embryo. After germ band retraction, staining is seen in the thorax and abdomen in segmentally repeated epidermal stripes which are one cell wide and are situated at the anterior border of each segment. These stripes are present both dorsally and ventrally in the abdomen, but are present only dorsally in the thorax. Staining is also seen in the posterior spiracles and in a loop of cells in the hindgut which extends from one posterior spiracle to the other. The staining pattern in the hindgut and posterior spiracles is shown in Figure 2F. The *hobo* enhancer trap staining pattern is very similar to the staining pattern shown by a *P* element enhancer trap at 62A in the gene *rhomboid* (BIER, JAN and JAN 1990). In this case it has been shown that the *P* element enhancer trap staining pattern recapitulates part of the later embryonic expression pattern of the gene beginning at germ band extension and continuing to later stages. The *hobo* insertion is homozygous viable but confers a recessive rough eye phenotype upon the flies. It may be that the *hobo* element is inserted in a gene adjacent to *rhomboid* and that the insertion into this gene is responsible for the eye phenotype, but that the *hobo* enhancer trap is nevertheless responding to enhancer elements in *rhomboid*. Alternatively, the eye phenotype may be an undescribed aspect of the genetics of *rhomboid*.

We have examined one case in some detail. The *hobo* enhancer trap of line 151 was inserted at 22F which is where the *dpp* gene is located. We used plasmid rescue (PIROTTA 1986) to isolate genomic DNA flanking the transposable element. Southern blot analysis using this DNA as a probe was employed to show that the transposon had inserted into the *disk* region of the *dpp* gene by hybridization to phage DNA from the region (ST. JOHNSTON *et al.* 1990). The position of the *hobo* insertion is shown in Figure 9. Interestingly, the enhancer trap had inserted close to the sites of insertion of two previously mapped independent *hobo* insertions into the gene (GELBART and BLACKMAN 1989). The enhancer trap staining pattern faithfully reproduced part of the later embryonic staining pattern of the *dpp* gene but did not mirror the earlier dorsal ectodermal expression patterns (ST. JOHNSTON and GELBART 1987; RAY *et al.* 1991). Previous studies (BLACKMAN *et al.* 1991) have shown that the *disk* region consists of 3′-regulatory sequences which regulate the *dpp* gene in *cis*. The BS3.0 DNA fragment extends from 106.9 to 116.9 in the *disk* region and encompasses the site of insertion of the *hobo* enhancer trap. When this DNA fragment was placed next to a β-galactosidase reporter gene and introduced into the fly genome, part of the embryonic *dpp* expression was reproduced (BLACKMAN *et al.* 1991) and the pattern of expression conferred by this fragment appears identical to that of the *hobo* enhancer trap. The BS3.0 fragment also confers an expression pattern upon a reporter gene in imaginal disks which represents part of the *dpp* expression pattern. The *hobo* enhancer trap expression pattern in disks is similar to the pattern conferred by BS3.0 (data not shown).

***P[ry⁺, HBL1]* can be used to make mutations in a gene next to a homozygous wild-type *hobo* enhancer trap insertion:** *hobo* transposase can cause imprecise excisions of a *hobo* insertion and such excisions can result in deletions of DNA flanking the insertion (BLACKMAN *et al.* 1987). We used this observation to

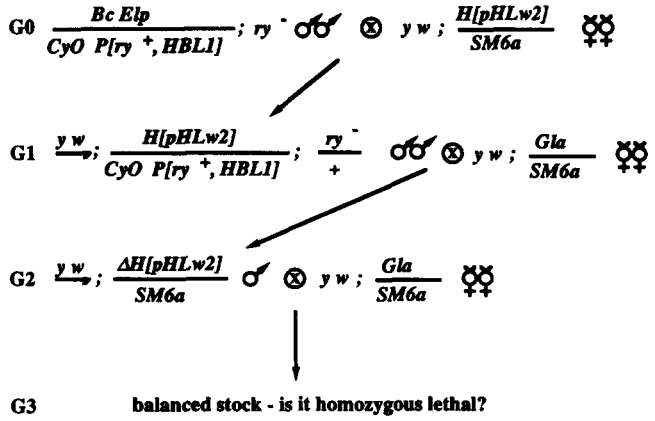


FIGURE 10.—Genetic crosses employed to produce mutations in the *dpp* gene by creating imprecise excisions of the *hobo* enhancer trap in line 151. Excisions of *H[pHLw2]* are denoted by *ΔH [pHLw2]*.

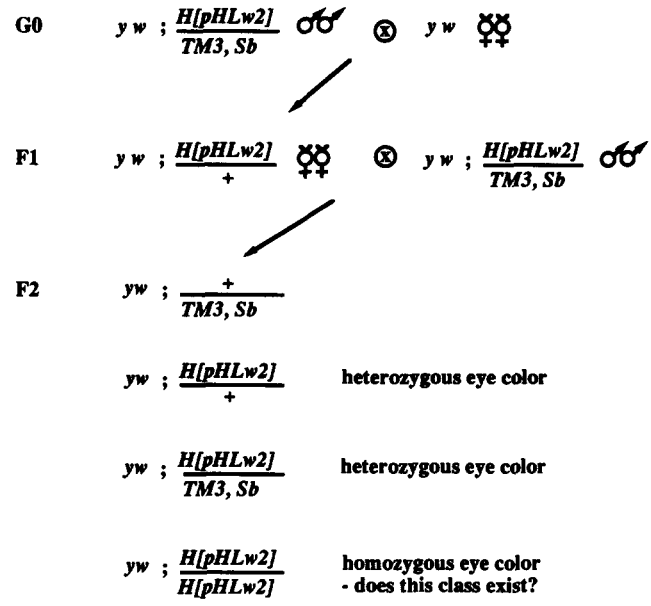


FIGURE 11.—Genetic crosses employed to obtain the meiotic recombination distances between the *hobo* insertions and the recessive lethal phenotypes.

generate mutations in the *dpp* gene using line 151 which is a homozygous viable insertion. The genetic crosses employed are shown in Figure 10. Males obtained in the G_2 generation with a different shade of w^+ eye color than heterozygotes from the parental line 151 were used to produce stocks balanced for the second chromosome. w^- flies presumably represent removal of the *hobo* insertion, whereas those flies which possess a darker shade of w^+ eye color than the parental line could represent a more complex event which could include, for example, a local jump. Of 72 fertile G_1 crosses that were set up, 6 gave rise to progeny with an altered shade of w^+ eye color. One set of exceptional progeny consisted of w^- sons and the other five consisted of sons with a darker shade of w^+ eye color than the parental line. Two of the balanced stocks established from these flies were homozygous lethal. One was w^- (line 151 event A) and the other had a darker shade of w^+ eye color than the parental line (line 151 event B). These stocks were tested in *trans* to three *dpp disk* alleles: dpp^{d19}, $dpp^{d\text{-}blk}$ and $dpp^{d\text{-}ho}$ (ST. JOHNSTON *et al.* 1990). Each insertion derivative failed to complement one of the alleles tested. Line 151 event A displayed the heldout phenotype in *trans* to $dpp^{d\text{-}ho}$. Line 151 event B had reduced wings in *trans* to dpp^{d19} and this phenotype is characteristic of *dpp* class II *disk* mutations.

Homozygous lethality: Of the 612 lines that were created, 503 were localized to a chromosome. Eight mapped to the *X* chromosome, 206 to the second chromosome and 281 to the third chromosome. Eight lines did not map to either the *X*, second or third chromosomes and these were presumed to map to the fourth chromosome or represent double insertions of the transposable element onto two different chromosomes. Of the lines mapping to the second and third chromosomes, 17% (35/206) and 28% (78/281), respectively, possessed a recessive lethal phenotype. We do not know why the rate of recessive lethal phenotypes is higher for inserts on the third chromosome compared to the second chromosome. In light of the experiments described below, it seems unlikely that this is due to undetected lethal mutations segregating on the third chromosome. The average rate of recessive lethal phenotypes on the second and third chromosomes is 23% (113/487). There was no correlation between whether a line displayed specific staining and whether it was a homozygous lethal insertion.

As mentioned above, it cannot be assumed that the homozygous lethality of a line is due to the enhancer trap insertion. For example, the screen was not performed in an isogenized background and there might be undetected lethal mutations segregating. In addition, it could be imagined that the source of *hobo* transposase might cause mutations that are not associated with the insertion of a w^+ enhancer trap. For example, in the case of *P* elements, BERG and SPRADLING (1991) speculated that some mutations may result from insertion followed by immediate imprecise excision of an element. The same speculation may be true for *hobo* elements.

What proportion of the homozygous lethal *hobo* lines are due to the transposon? Two approaches were used to answer this question, employing *hobo* insertions on the third chromosome. The first approach used recombinational mapping to attempt to separate the *hobo* insertion from the recessive lethal phenotype. The genetic crosses that were used are shown in Figure 11. The approach exploits the property of the *mini-white* marker gene mentioned earlier (see materials and methods) which means the gene confers a darker w^+ eye color upon flies homozygous for the marker compared to heterozygous flies. Since

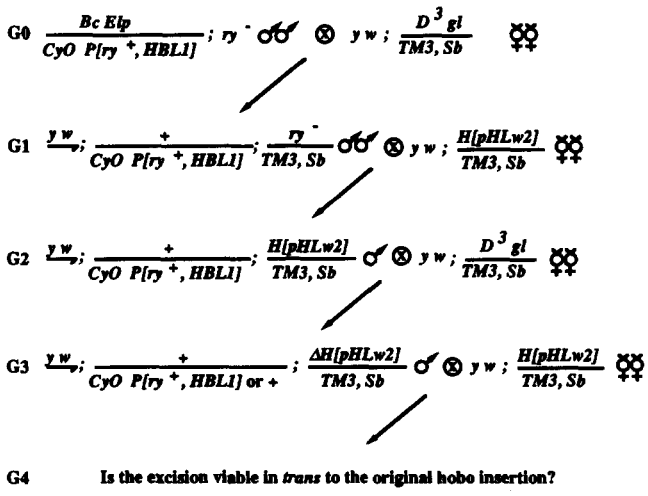


FIGURE 12.—Genetic crosses employed to obtain revertants of the recessive lethal phenotype by excision of the *hobo* insertions. Excisions of *H[pHLw2]* are denoted by *ΔH[pHLw2]* and were recognized by loss of the *mini-white* marker leading to a w^- eye color in the G_3.

the w^+ eye color produced by the *mini-white* marker gene becomes darker as the fly ages, the F_2 generation were scored every 2 days in order to minimize variation due to this cause. For each line, at least 1,500 F_2 flies were scored. Failure to observe individuals in the F_2 generation that were homozygous for the *hobo* enhancer trap would imply that the recessive lethal phenotype maps to within 0.8 cM ($\chi^2 = 4$, 1 d.f., $P < 0.05$) of the *hobo* insertion and hence that the insertion event is probably responsible for the phenotype. Confirmation that F_2 flies were homozygous for the *hobo* enhancer trap was obtained by outcrossing representative individuals to *y* w^{67c23} flies and observing that all the offspring were w^+. For 17 out of 20 lines examined (17/20 = 85%) it was not possible to separate by recombination the *hobo* insertion from the recessive lethal phenotype. This suggests that for the majority of recessive lethal *hobo* insertions that the phenotype is due to the transposable element.

The second approach employed the stable source of transposase *P[ry⁺, HBL1]* to excise the *hobo* enhancer trap using the genetic crosses shown in Figure 12. If the transposable element is responsible for the recessive lethal phenotype, then the phenotype may be revertable by excision of the element. Excisions were recognized by loss of the *mini-white* marker leading to a w^- eye color in the G_3. Fifteen lines were examined using this approach and the results are shown in Table 4. Thirteen lines yielded excisions and of these, nine were rendered viable over the original lethal *hobo* insertion. Thus, this approach again suggests that for the majority of lethal *hobo* insertions (9/13 = 69%), the lethality is due to the insertion. The evidence provided by both approaches makes it seem likely that most of the recessive lethal mutations are caused by the *hobo* insertion.

TABLE 4

Reverting recessive lethal phenotypes by excision of the *hobo* enhancer trap

Line	No. of G_2 crosses[a]	No. of G_2 crosses yielding excisions[b]	No. of independent excisions viable over the original *hobo* insertion[c]
42	37	6	0
128	45	8	4
158[d]		≥1	≥1
172	50	0	
178	47	3	3
189	31	2	2
190	41	1	0
314	35	0	
339	46	4	3
401	45	1	0
408	45	6	5
422	48	1	0
423	42	4	3
536	37	2	1
549	40	2	1

[a] Number of fertile G_2 crosses.
[b] Number of crosses producing at least one son with an excision.
[c] Number of crosses producing at least one son with an excision that is viable over the original *hobo* insertion. Sometimes sibling males from the same cross were independently tested. These males could represent the same pre-meiotic event. They gave the same result in all cases.
[d] This line has 5% escapers. The escapers have abnormal morphology with downturned wings and double bristles emerging from single sockets. This line was tested by directly crossing 10 G_2 males *en masse* to tester females containing the *hobo* insertion and screening 750 progeny for males lacking the balancer chromosome or for males containing w^- excisions over balancer. The second class of male was then tested by crossing them singly to females containing the hobo insertion. The homozygous revertants display normal morphology.

Comparison of *hobo* and *P* insertion patterns on chromosome *3*: The 122 *hobo* insertions localized on chromosome *3* were compared with a compilation of single *P* element insertion sites (MERRIAM *et al.* 1991) using lettered subdivisions as the unit of comparison. The *hobo* insertions were derived from 118 lines mapping to chromosome *3*. Entries in the *P* element compilation were only included in the analysis if they were localized within at most two lettered subdivisions. The small proportion of entries localized for example as 93A/B were treated as being localized to 93A for the purpose of the analysis. There were a total of 619 *P* element insertions used for the analysis.

Table 5 shows the distribution of the number of *hobo* elements per lettered subdivision. If each subdivision was equally likely to receive an insertion, the number of insertions would be expected to be Poisson distributed; this is clearly not the case, as the test for goodness of fit shows. If, on the other hand, subdivisions differ in the probability of insertion, many functional forms for the distribution are possible. Of those examined, the negative binomial provides the best fit. Similarly, the distribution of the number of *P* inser-

TABLE 5

Distribution of the number of *hobo* elements per lettered subdivision on the third chromosome

N	Observed	Expected Poisson	Expected negative binomial
0	159.00	139.68	160.33
1	51.00	72.82	46.44
2	12.00	18.83	16.99
3	9.00	3.22	6.65
4	1.00	0.41	2.69
5	2.00	.04	1.11
≥6	1.00	0.00	0.78
Goodness of fit:	*G*	29.98	4.85
	d.f.	3	4
	P	<0.0001	<0.303

TABLE 6

Distribution of the number of *P* elements per lettered subdivision on the third chromosome

N	Observed	Expected Poisson	Expected negative binomial
0	45.0	16.78	43.66
1	50.0	44.38	49.77
2	34.0	58.60	42.48
3	37.0	51.50	32.20
4	29.0	33.90	22.88
5	17.0	17.82	15.60
6	7.0	7.79	10.34
7	2.0	2.92	6.71
8	7.0	0.95	4.29
9	0.0	0.28	2.70
10	3.0	0.07	1.69
≥11	4.0	0.02	2.32
Goodness of fit:	*G*	91.64	18.97
	d.f.	7	10
	P	<0.0001	<0.041

tions (Table 6) is not Poisson but shows a better fit to a negative binomial, giving some confidence that the *P* and *hobo* samples are comparable. There is more subdivision to subdivision variability in the *P* sample than in the *hobo* sample; this may be due to the heterogeneous nature of the sample or to a greater instrinsic variability in *P* insertion sites.

Table 7 shows the joint distribution of *P* and *hobo* elements for each subdivision. A contingency χ^2 test (ROHLF and SOKAL 1981; SOKAL and ROHLF 1981) was performed on a condensed version of this table, pooling classes with two or more *hobo* elements and six or more *P* elements. The condensed table has a sufficiently high expectation in each cell to carry out the test. The test examines whether there is any correlation between the likelihood of lettered subdivisions being hit by *P* or *hobo* elements and could, for example, reach significance if hot spots for *P* and *hobo* insertion tended to be the same. The null hypothesis is that there is no tendency for *P* and *hobo* elements

TABLE 7

An *R*×*C* table comparing the occupancy of lettered subdivisions for *P* and *hobo* elements on the third chromosome

	hobo occupancy							
P occupancy	0	1	2	3	4	5	6	Total
0	35	7	1	2	0	0	0	45
1	37	10	2	0	0	0	1	50
2	21	7	2	3	1	0	0	34
3	25	9	2	1	0	0	0	37
4	15	10	3	1	0	0	0	29
5	12	3	0	1	0	1	0	17
6	4	2	0	1	0	0	0	7
7	2	0	0	0	0	0	0	2
8	3	1	2	0	0	1	0	7
9	0	0	0	0	0	0	0	0
10	2	1	0	0	0	0	0	3
11	2	0	0	0	0	0	0	2
12	0	1	0	0	0	0	0	1
13	1	0	0	0	0	0	0	1
Total	159	51	12	9	1	2	1	235

to be found in the same subdivisions. The test was not significant ($\chi^2 = 10.75$, 12 d.f., $P < 0.55$) and hence there is no evidence of any association between the insertion sites of the two elements. In order to remove any biases that may have arisen in the sample of *P* elements, a test was also carried out on a table in which *P* elements have been recorded as present or absent. Again, there was no evidence of association.

Additional statistical tests were carried out to examine the distributions of *P* and *hobo* insertions along the length of the third chromosome. A Kolmogorov-Smirnov test (ROHLF and SOKAL 1981; SOKAL and ROHLF 1981) showed that both distributions were significantly non-uniform ($D = 0.0922$, $P < 0.00005$ for *P* elements and $D = 0.1555$, $P < 0.0055$ for *hobo* elements). More importantly, this test also showed that to a significant level ($D = 0.1591$, $P < 0.0115$) the two transposons were distributed differently with respect to each other. The last result was confirmed by dividing the chromosome into 20 regions of two numbered divisions each, proceeding from the left telomere to the right telomere of the chromosome. The number of *P* and *hobo* elements was compared in each region using the χ^2 test. The test was significant ($\chi^2 = 40.3$, 19 d.f., $P < 0.003$). The distributions of *P* and *hobo* inserts along chromosome *3* are illustrated in Figure 13 in which the distributions are smoothed out for ease of visual inspection. The statistical analyses were performed using the unsmoothed data. Figure 13 demonstrates that *P* elements tend to be clustered in the proximal region of the right arm, while the *hobo* elements tend to be found in the more distal locations of each arm.

DISCUSSION

Comparison of *hobo* and *P* element enhancer trapping: One of the prominent features of the *hobo*

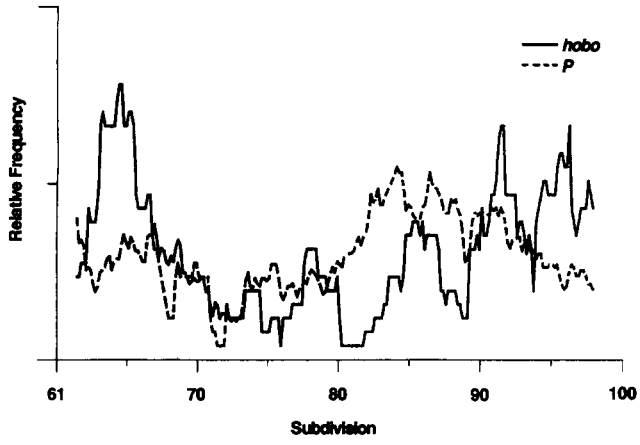


FIGURE 13.—Distributions of *P* and *hobo* elements along chromosome *3* smoothed using a sliding window of 15 lettered subdivisions. The sizes of the distributions have been normalized with respect to each other and the units of the ordinate are therefore arbitrary.

enhancer trap screen is that the most frequently staining tissues are the nervous system and the gut. This is very similar to what is observed using *P* element enhancer traps. It was suggested based on the *P* element enhancer trap data that this predominance may reflect the possibility that the nervous system and the gut displays the largest complexity of cell types (BELLEN *et al.* 1989; BIER *et al.* 1989). This suggestion may explain the similar staining profiles that are seen using the different transposable element system described here.

It has been shown for many *P* element enhancer traps that the staining pattern of the enhancer trap faithfully reproduces part or all of the expression pattern of a nearby gene. It seems likely that the same will hold true for the hobo enhancer trap: three examples are discussed in the present report where this seems to be the case. In addition, in a manner similar to what has been shown for *P* elements, we have demonstrated that it is possible to use *P[ry*$^+$*, HBL1]* to create imprecise excisions of a viable hobo insertion and thereby create mutations in a nearby gene. Further, we have shown that most of the recessive lethal phenotypes are due to *hobo* insertion.

Using single *P* element mutagenesis, the rates of recessive lethal phenotypes for the second and third chromosomes were 17% and 11%, respectively, in one study (BIER *et al.* 1989) and 10% and 11%, respectively, in another (COOLEY, KELLEY and SPRADLING 1988). These rates have proven fairly typical for other single *P* element mutageneses.The rates of recessive lethal phenotypes for the second and third chromosomes using the hobo enhancer trap were higher, 17% and 28% respectively. Whether this discrepancy is due to the use of two different transposons remains to be seen. Many *P* element alleles prove to be hypomorphs and this may be due to the well established tendency of *P* elements to insert near the transcriptional initiation site of genes rather than into coding sequences. It may be that *hobo* elements do not display this tendency. This issue could be resolved by investigation of the insertion sites of a number of lethal hobo insertions. In addition, it has been suggested that the ability of a transposon to cause mutations may depend on the sequences contained within it (BERG and SPRADLING 1991) and it may be that the sequences contained within the *hobo* enhancer trap are partially responsible for its higher mutation rate. In any event, the general issue of the rate of recessive lethal phenotypes produced by single element *hobo* mutagenesis may repay further study.

Comparison of *hobo* and *P* insertion specificities: One approach to analysis of the mutation preferences of the two transposon systems would be to attempt a single element mutagenesis of chromosome *3* (for example) with both *hobo* and *P* elements and then to compare the frequency with which various loci are mutated by each element. Such an approach would probably be impracticable, since the number of loci on chromosome *3* mutable to lethality by chemical mutagens is estimated to be about 1650 (JURGENS *et al.* 1984) and the number readily mutable to lethality by *P* elements has been estimated to be at least 582 (COOLEY, KELLEY and SPRADLING 1988). Thus, the potential number of targets for insertion is so large that it is unlikely that it would be possible to make a convincing argument that the distributions of the two elements in general are different, though this approach might provide useful information about hot spots.

We have therefore chosen to take a different approach. We have localized a number of independent *hobo* enhancer trap insertions on the third chromosome and compared these with a compilation of *P* element insertions. The *P* element insertions in the compilation have many different origins and it is difficult to address whether the mode of production of a *P* element insertion affects its insertion specificity. However, BERG and SPRADLING (1991) found no significant difference between the two populations of insertional mutants on chromosome *3* produced by two separate *P* elements at different positions on the *X* chromosome. The compilation of localized *P* element insertions depends on the generosity of the fly community, and there is no evidence that it has been subjected to systematic sampling bias (MERRIAM *et al.* 1991). The localized *hobo* insertions were a representative sample of the whole collection, although lethal lines were somewhat underrepresented (20/118 = 17%) and specifically staining lines were somewhat overrepresented (71/118 = 60%).

The $R \times C$ contingency χ^2 test revealed no association between *P* and *hobo* elements at the lettered

subdivision level and the Kolmogorov-Smirnov test indicated gross differences at the chromosomal level. Of the two tests, the Kolmogorov-Smirnov test is more probably more reliable in this context. The $R \times C$ contingency χ^2 test compares the two distributions at the level of the lettered subdivision and is therefore susceptible to error if there are inaccuracies in cytology in either distribution. The Kolmogorov-Smirnov test compares more global aspects of two distributions and hence is much less sensitive to inaccuracies at the lettered subdivision level. In addition, the test is nonparametric and conservative (ROHLF and SOKAL 1981; SOKAL and ROHLF 1981). In the comparison of the *P* and *hobo* distributions, the test was significant at close to 1%. The different insertion patterns for *P* and *hobo* may reflect a different distribution of local hot spots for the two elements or other large scale regional preferences.

A genetic approach toward comparing *hobo* and *P* element insertion specificity may be possible using local jumping which has recently been described for *P* elements (TOWER *et al.* 1993; ZHANG and SPRADLING 1993) and which we and others (SHEEN, LIM and SIMMONS 1993) suggest may also occur for *hobo* elements. This approach may allow extensive mutagenesis of a genetic region and permit further comparison between the insertion preferences of the elements. More generally, perhaps one of the most important tasks in the future development of *hobo* insertional mutagenesis is to improve the jump rate. This would improve the usefulness of the system and would decrease the labor involved in future investigations. We have shown in this report that a recombinant chromosome containing two *hobo* elements provides a higher jump rate than chromosomes containing a single element. More substantial improvements might be obtained by the development of stronger sources of *hobo* transposase.

In conclusion, this report shows that the *hobo* element can be used effectively for enhancer trap mutagenesis. In addition, the present data shows *P* and *hobo* elements have different insertion specificities and hence *hobo* insertional mutagenesis and enhancer trapping should be valuable additional tools for more complete coverage of the Drosophila genome.

We thank Y. HIROMI for pFZ and V. PIRROTTA for pCahsneominiwhite. We thank Y. HIROMI and R. W. PADGETT for advice on construction of pHLw2, M. FRISARDI for teaching D.S. how to do *in situ* hybridization, KEVIN LEE and CLIVE WILSON for advice on nervous system staining, PEI-HSIN HUANG and MICHELE SANICOLA for performing the Southern blot analysis of phage from the *disk* region of *dpp* and J. LISTER and K. MYERS for technical assistance in the early stages of this project. D.S. is grateful for support from a Serc/NATO research fellowship and the Horace Le Marquand and Dudley Bigg research fellowship of the Royal Society. B.R.C. was supported by a National Science Foundation predoctoral fellowship and a U.S. Public Health Service predoctoral traineeship in genetics. This work was supported by a National Institutes of Health grant to W.M.G.

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Communicating editor: P. CHERBAS