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Targeted Transposition at the *vestigial* Locus of *Drosophila melanogaster*

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ABSTRACT

Targeted transposition is the replacement of one *P* element with another. We are exploiting this unique property of *P* elements to study the complex regulatory domain of the *Dopa decarboxylase* (*Ddc*) gene in *Drosophila melanogaster*. *P* element constructs targeted to the same site in the genome will be subjected to the same position effect. This allows the subtle effects typical of most mutations in the *Ddc* regulatory region to be measured in the absence of the variable influences of position effects which are associated with the current method of germline transformation. We have investigated some of the parameters affecting targeted transposition of a *Ddc* transposon, *P*[*Ddc*], into a *P* element allele at the *vestigial* locus. These events were detected by an increased mutant *vg* phenotype. The location of the donor transposon in *cis* or in *trans* to the target had little effect on the frequency of targeting. Likewise, the mobility of different donor elements, as measured by their rate of transposition to a different chromosome, varied nearly 20-fold, while the rate of targeted transposition was very similar between them. All targeted alleles were precise replacements of the target *P* element by *P*[*Ddc*], but in several cases the donor was inserted in the opposite orientation. The targeted alleles could be described as the result of a replicative, conversion-like event.

P-M hybrid dysgenesis is a syndrome in *Drosophila melanogaster* associated with temperature-sensitive gonadal sterility, pupal lethality, increased mutation rate and male recombination (KIDWELL *et al.* 1977). It is caused by *P* elements (BINGHAM *et al.* 1982; RUBIN *et al.* 1982), a family of transposable elements which includes the autonomous 2.9-kb element and internally deleted non-autonomous elements (O'HARE and RUBIN 1983).

Experimentation on the regulation of gene expression in *D. melanogaster* was revolutionized by the advent of *P* element transformation (RUBIN and SPRADLING 1982; SPRADLING and RUBIN 1982). However, transformation studies are complicated by "position effects," the dependency of expression of a transgene on its site of insertion in the genome (SCHOLNICK *et al.* 1983; SPRADLING and RUBIN 1983; CHEN and HODGETTS 1987). Transcription of the gene *Dopa decarboxylase* (*Ddc*) in the epidermis is dependent on a set of partially redundant, *cis*-acting regulators (HIRSH *et al.* 1986; SCHOLNICK *et al.* 1986). Each regulator has relatively small additive effects which are similar in magnitude to position effects making germline transformation difficult to use in the analysis of *Ddc*, whose regulation we are studying.

We have been developing a technique, that we refer to as targeted transposition (HESLIP *et al.* 1992), to circumvent the problem of position effects on transgene expression caused by the random nature of *P* element insertions. Targeted transposition is the replacement of a *P* element (the target) by another *P* element (the donor). The replacement is precise, leaving the local genomic DNA unchanged. This event can result in an increase in mutant phenotype as reported at the *P* element induced hypomorphic alleles of the *vestigial* and *yellow* loci, vg^{21} and $y^{13\text{-}11}$ (WILLIAMS *et al.* 1988; GEYER *et al.* 1988).

Using the enhanced mutant phenotype of *vg* as the basis of a targeting scheme, a *P* element transposon carrying the *Ddc* gene, *P*[*Ddc*], and one carrying an enhancer trap have been recovered at *vg* (HESLIP *et al.* 1992; STAVELEY *et al.* 1994). We undertook the present study to assess various parameters that might influence the rate of targeted transposition. The effect of the location of the donor transposon in a *cis* or *trans* configuration relative to the target, vg^{21} was examined. We also measured the transposition rate of different donor transposons to determine if there is a relationship between the mobility of a donor transposon and the rate of targeting. Our analyses indicate that all targeted alleles result in precise replacement of the resident vg^{21} *P* element and that most contain an internally deleted *P*[*Ddc*]. In general, donor location has little influence on the rate of transposition. However, when the donor resides on the target's homologous chromosome, the rate of targeting is very low.

MATERIALS AND METHODS

***D. melanogaster* stocks:** All crosses were performed at 22–24° unless otherwise noted on yeast medium (NASH and BELL 1968). Ddc^{ts2} and Ddc^{n7} are temperature-sensitive and recessive null alleles, respectively, of the *Ddc* gene (WRIGHT *et al.* 1981). The vg^{21} allele is a *P* element insertion into the *vg* locus and vg^{79d5} is caused by a small deletion within the locus (WILLIAMS and BELL 1988). The third chromosomes, *TM2, Ubx ry P*[ry^+ Δ2-3] (99B) and *Sb* ry^{506} *P*[ry^+ Δ2-3] (99B), contain a stable genomic source of transposase (ROBERTSON *et al.* 1988)

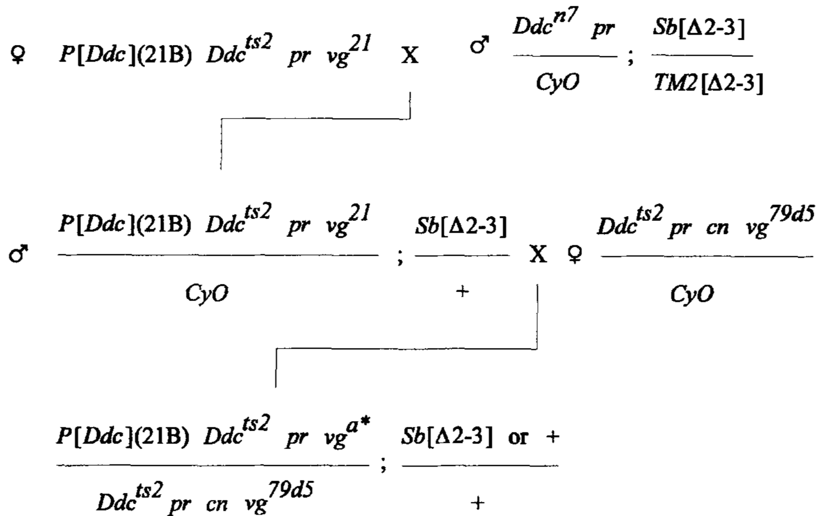


FIGURE 1.—Crossing scheme for targeting in *cis*. Males and females are indicated. The targeting crosses are designed to target *P*[*Ddc*], located at 21B, into vg^{21}. This occurs in the germline of the males in the second cross. The progeny of the second cross are raised at 29°. Those progeny inheriting the putative targeted allele, vg^{a*}, are detected by their more extreme mutant *vg* phenotype and used to establish stocks. The *CyO* second chromosome is marked with *cn* and *pr*. The *TM2* third chromosome is marked with *Ubx* and *e*.

and will be referred to as *TM2*[Δ2-3] and *Sb*[Δ2-3], respectively. The donor, *P*[*Ddc*], is a *P* element vector, pHDlac, containing a 7.6-kb *Pst*I fragment that includes the *Ddc* gene (CHEN and HODGETTS 1987). The *P* element donor carried on the *X* chromosome, *P*[inv*Ddc*], contains the same 7.6-kb *Pst*I *Ddc* fragment as *P*[*Ddc*] but in the opposite orientation relative to the *P* element vector sequences (MARSH *et al.* 1985). Other mutant alleles and chromosomes are described in (LINDSLEY and ZIMM 1992). The targeting crosses (see Figures 1, 3 and 4) were conducted batchwise in bottles. The occurrence of targeted transpositions was not clustered; therefore, premeiotic events resulting in more than one targeted transposition can be ruled out.

DNA manipulations: Culturing and storage of bacteria, preparation of plasmid DNA, and restrictions were performed according to standard methodology (MANIATIS *et al.* 1982). *D. melanogaster* genomic DNA for Southern hybridization was isolated as in HESLIP *et al.* (1992).

Genomic DNA to be used for DNA amplification was isolated as follows. Flies were homogenized in 50 μl of 10 mM Tris-Cl, pH 7.5, 60 mM EDTA, 0.15 mM spermidine, 0.15 mM spermine, and 100 μg/ml proteinase K. After incubating the homogenate for 30–60 min at 37°, 50 μl of 0.2 M Tris-Cl, pH 9.0, 30 mM EDTA, 2% sodium dodecyl sulfate (SDS) were added and incubated at 65° for 30 min with occasional agitation. A single organic extraction was performed; 150 μl of 1:1 phenol/chloroform was added, mixed and warmed to 65°. After brief centrifugation, 80 μl of the aqueous phase were subjected to DNA purification using Geneclean II (Bio 101 Inc.) according to the manufacturer's instructions. The purified DNA was resuspended in 30 μl of TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0). Genomic DNA was extracted from single flies that were used to establish lines (see RESULTS). Flies with a borderline increase in mutant *vg* phenotype were pooled using 2–4 individuals in a single DNA extraction.

Southern hybridizations: Restricted DNA was fractionated by agarose gel electrophoresis and transferred onto Genescreen Plus membranes (DuPont). Hybridizations were performed at 42° in 50% formamide according to the manufacturer's instructions using oligolabeled DNA restriction fragments (FEINBERG and VOGELSTEIN 1983) purified from agarose gels with Geneclean.

DNA sequencing: DNA amplification products were purified from agarose gels with Geneclean and sequenced using the ABI Taq DyeDeoxy Terminator Cycle Sequencing Kit according to the manufacturer's instructions. The sequencing reactions were fractionated and analyzed on an ABI 370A DNA sequencing apparatus.

DNA amplification: Each amplification reaction used 3 μl (approximately 50 ng) of DNA in a total volume of 30 μl which contained 2–3 units of Taq polymerase (Bio-Can or BRL) and a final concentration of 50 mM Tris-Cl (pH 9.2), 1.5 mM $MgCl_2$, 0.005% β-mercaptoethanol, 0.1 μg/μl bovine serum albumin (Boehringer Mannheim), 200 μM of dATP, dCTP, dGTP, dTTP, and 0.3 ng/μl of primer. Reactions were run in a Stratagene Robocycler 40. The amplification program started with 5 min at 95°, 1.5 min at 60°, and 3 min at 73°, followed by 29 cycles of 1 min at 93°, 1 min at 60°, and 2–3 min at 73°. All amplification products were run on agarose minigels at 70 V. Primers used are listed by numbers as they appear in the results: #1; 5′-ATCCCGCGCGGCGGTGAGAG-3′, #2; 5′-TTCGGAGCGTGATGTTGACA-3′, #3; 5′-ATCGGCGTTGTA-AAGACTGC-3′, #4; 5′-GTACTCCCACTGGTATAGCC-3′, #5; 5′-CGTCGAAAGCCGAAGCTT-3′, #6; 5′-AATCAAGTGGG-CGGTGCTTG-3′.

RESULTS

Targeting in a *cis* configuration: In our previous study the donor and the target were on homologous chromosomes in a *trans* configuration (HESLIP *et al.* 1992). We obtained one targeted *vg* allele, vg^{28w}, out of 18,500 flies scored. This low frequency led us to examine whether targeted transposition would occur faster if the donor and the target were in a *cis* configuration. To test this, the crosses shown in Figure 1 were set up to target the *P*[*Ddc*] at 21B into vg^{21} at 49D on the same chromosome. The germline of the males in the second cross contains the target (vg^{21}), the donor transposon (*P*[*Ddc*]), and the transposase source ([Δ2-3]). Some of the progeny from these males could contain a targeted *vg* allele, denoted as vg^{a*} in Figure 1. The increase in mutant *vg* phenotype, indicative of a targeted event, is easily scored in the vg^{79d5} heterozygote, since vg^{21}/vg^{79d5} exhibits a weak phenotype. Any fly with a stronger mutant phenotype than vg^{21}/vg^{79d5} was mated to Ddc^{ts2} *pr cn* vg^{79d5}/CyO flies to establish a line over the *CyO* balancer chromosome. Once a viable culture of larvae was

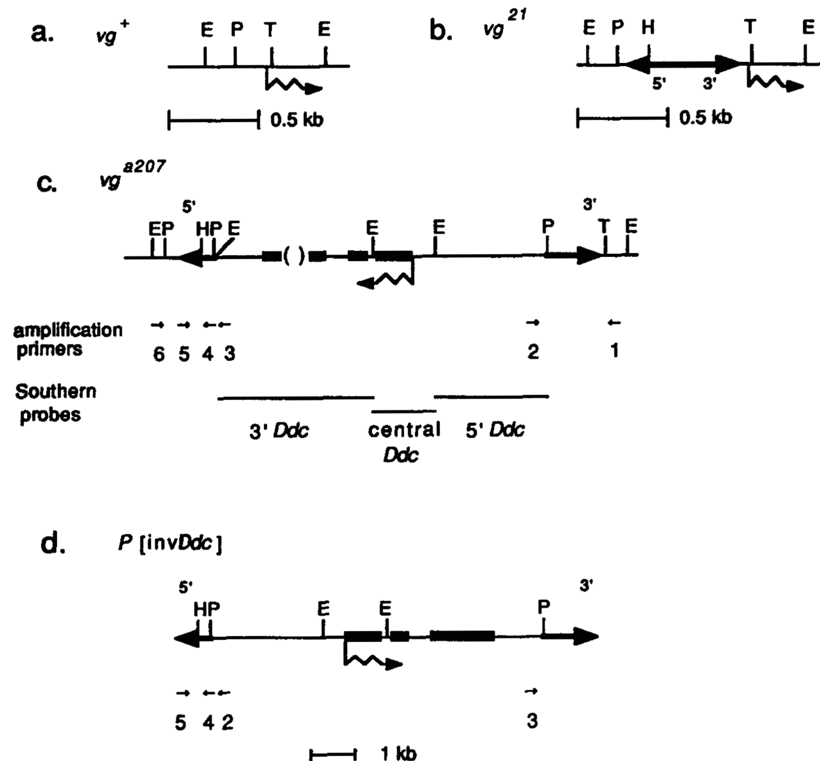


FIGURE 2.—Restriction maps of the *vestigial* alleles in this report. Restriction sites are as follows: E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; T, *Sst*I. All *Eco*RI sites are shown but only informative sites are shown for the rest, including the characteristic *Hin*dIII at the 5′ end of *P* elements. (a) The vg^+ allele. The vg^+ probe referred to in the text is the 0.7 kb *Eco*RI fragment from this gene. The wavy arrow indicates the 5′ end of the first exon of *vg* (WILLIAMS *et al.* 1990). (b) The vg^{21} allele. The 5′ and 3′ ends of the *P* element are indicated below the solid arrows (↔) on the map. The 3′ end of the *P* element is about 20 bp upstream of the first exon of *vg* (WILLIAMS *et al.* 1992). (c) The vg^{a207} allele. The 5′ and 3′ ends of the *P* element vector of *P*[*Ddc*] are shown above the map. The wavy arrow indicates the transcription start site of *Ddc* and therefore the polarity of the *Ddc* gene is opposite to that of the *P* element. The putative *vg* transcription start site is not shown but remains unchanged from (b). Restriction map symbols: *vg* genomic DNA, ——; *P* element DNA with a terminus, →; *Ddc* exons and introns, -■-. The internal deletion of the *Ddc* gene in vg^{a207} is arbitrarily indicated by the round brackets. Below the restriction map are the locations and polarity (5′ to 3′) of the amplification primers whose sequences are listed in the MATERIALS AND METHODS. Below the primers are the locations of probes for Southern hybridizations. (d) The *P*[inv*Ddc*] donor. The orientation of the *P* element vector is the same as in (c); the 5′ and 3′ ends are shown above the map. The *Ddc* gene is cloned into this construct in the opposite direction compared to the *P*[*Ddc*] shown in (c). The restriction map symbols are the same as in (c). The location of amplification primers is shown below the map.

established, the founding adult was frozen and used for single fly DNA amplification. These crosses and subsequent analyses constitute experiment a.

Molecular maps of vg^+ and vg^{21} are shown in Figure 2, a and b. The cryptic vg^{21} allele is caused by the insertion of a 687-bp *P* element just upstream of the putative transcription start site (WILLIAMS *et al.* 1992). Figure 2c illustrates the molecular map of a typical targeted allele, vg^{a207}. The three primers used for single fly DNA amplification are also shown. These oligonucleotides are complementary to downstream *vg* (#1), 5′ *Ddc* (#2), and 3′ *Ddc* (#3) sequences, respectively. Following the amplification reaction, a product will be observed only in flies bearing a targeted transposition. Using both *Ddc* primers and the *vg* primer ensures that a product will be seen regardless of the orientation of the *P*[*Ddc*] in a targeted allele. A total of 8940 flies was scored from experiment a. Genomic DNA from 221 flies with an increased mutant *vg* phenotype plus approximately 150 flies with borderline phenotypes was subjected to amplification. Table 1 shows that three targeted alleles, vg^{a43}, vg^{a202} and vg^{a207}, were recovered. This rate of targeted transposition was roughly sixfold higher than that found in our previous experiment using the same *P*[*Ddc*](21B) donor.

Targeting in *cis* using a more proximal donor: In the targeted gene replacement experiments of GLOOR *et al.* (1991), the highest rate of conversion at the *white* locus was obtained using the most proximal donor template. To test the effect of a more proximal donor on targeted transposition, we carried out the crosses shown in Figure 3 in which the donor *P*[*Ddc*] was located at 35C. The progeny from the second cross were scored for an increase in mutant *vg* phenotype and targeted alleles were confirmed using the same procedure as in experiment a. The data are grouped as experiment f in Table 1. Three targeted alleles, vg^{f38}, vg^{f49} and vg^{fs1} were detected amongst the 320 flies that we analyzed with a borderline

TABLE 1

DNA amplification of targeted alleles

Experiment	Line	Amplification reaction primers[a] #1, #2 and #3	#1 and #4	#4 and #6	No. of flies scored
a	a43	+	–	+	8940
	a202	+	–	+	
	a207	+	–	+	
f	f38	+	–	+	6773
	f49	+	+	–	
	fs1	+	–	+	
x	x335	+	–	+	5565
	x336	+	+	–	
	x347[b]	+	+	–	

[a] The reactions including primers #1, #2 and #3 were used in the initial screen for targeted alleles while the next two reactions using primers #1 and #4, and primers #4 and #6 were used to determine the orientation of donor in targeted alleles.

[b] The founding parent of line ×347 was used for these amplification reactions. When sublines ×347.2 and ×347.11, which were derived from ×347 (see *Southern analysis*), were analyzed, the same results were obtained (data not shown).

to clearly mutant phenotype after scoring 6773 flies. This rate is comparable to experiment a and suggests donor proximity has little influence on the rate of targeted transposition.

Targeting in *trans* from the *X* chromosome: As mentioned above, in our first experiment the donor *P*[*Ddc*] was located on chromosome *II*, in *trans* relative to vg^{21}, and the rate of targeted transposition was very low (HESLIP *et al.* 1992). To test the generality of this result, we set up a different *trans* experiment using a donor on the *X* chromosome. The *Ddc* gene contained in the *X*-linked donor (Figure 2d) was cloned into the *P* element vector in the opposite orientation compared to *P*[*Ddc*] (MARSH *et al.* 1985). For this reason, the *X*-linked donor will be referred to as *P*[inv*Ddc*]. In experiments a and f, the targeting was performed over the multiply inverted *CyO* chromosome. In order to be consistent, the targeting from the *X* chromosome was also carried out in a vg^{21}/CyO heterozygote. The crosses shown in Figure 4a were used to target *P*[inv*Ddc*] from 10C on the *X* chromosome into vg^{21}. The data are grouped as experiment x in Table 1. We tested 150 flies with an increase in mutant *vg* phenotype plus an additional 100 flies with a borderline phenotype. Table 1 shows that three targeted alleles were generated in experiment x from 5565 flies scored. Thus, this *trans* configuration of this donor yielded a rate of targeting similar to those observed from *cis* donor locations.

Targeting in *trans* from the homologous second chromosome: We did not utilize DNA amplification in the screening process of our first targeting experiment (HESLIP *et al.* 1992). Using DNA amplification, faster screening and consequently the testing of more flies with a slight increase in mutant phenotype is possible. Therefore, the original *trans* experiment was repeated to determine whether or not we might have overlooked targeted events by selecting only strong *vg* adults. The same crosses were used as in Figure 2a of HESLIP *et al.* (1992) the last of which is shown in Figure 4b. We tested DNA from approximately 100 flies with an obvious increase in mutant *vg* phenotype plus another 100 flies with borderline phenotypes. Out of 6627 flies scored no targeted transposition was detected.

The orientation and sequencing of targeted donors: The primary DNA amplification reaction used to detect targeted transposition, which included primers #1, #2 and #3 (Figure 2c), could discriminate between the possible orientations of a targeted donor based on the size of the amplification product. For example, when using *P*[*Ddc*] as the donor, if the *P* element portion of this transposon is inserted at *vg* in the same orientation as the original *P* element in vg^{21} then primers #1 and #2 will yield a 0.8-kb product. If *P*[*Ddc*] inserts in the opposite orientation then primers #1 and #3 will yield a 0.7-kb product. Similarly, when using *P*[inv*Ddc*], the relative orientation of a targeted donor can be determined based on the size of the product in the primary amplification reaction.

Products from the primary DNA amplification reactions suggested that the *vg* alleles *a43*, *a202*, *a207*, *f38*, *fs1* and *x335* contained donor *P* elements in the same orientation as the original vg^{21} *P* element and that *vg* alleles *f49*, *x336* and *x347* contained donor *P* elements in the opposite orientation as the original vg^{21} *P* element. To confirm the orientation of all targeted donor elements, primer #4 and either primer #1 (the downstream *vg* primer in Figure 2c) or primer #6 (the upstream *vg* primer shown in Figure 2c) were used in separate DNA amplifications of each targeted line. Primer #4 is complementary to *P* element sequences present in the donors but absent in the vg^{21} *P* element and directs synthesis toward the 5′ *P* element terminus. In these reactions we expect a product in only one of the two reactions carried out on each line. A product from primers #4 and #6 indicates that the targeted *P* element is in the same orientation as the original vg^{21} *P* element whereas a product from primers #4 and #1 indicates the targeted *P* element is in the opposite orientation. The results are shown in Table 1. The orientation of targeted donors in *vg* alleles *a43*, *a202*, *a207*, *f38*, *fs1* and *x335* is the same as the original vg^{21} *P* element. The orientation of the targeted donors in *vg* alleles *f49*, *x336* and *x347* is opposite to the original vg^{21} *P* element.

The DNA amplification products from the reactions using primers #4 and #6 on vg^{a43}, vg^{a202}, vg^{a207}, vg^{f38}, vg^{fs1} and vg^{x335}, were purified from agarose gels and sequenced. We have previously used a sequence polymorphism that exists in *P* element vectors at nucleotide position 33 (O'HARE and RUBIN 1983; RUBIN and SPRADLING 1983) to demonstrate the replacement of the 5′ end of the vg^{21} *P* element with the 5′ end of *P*[*Ddc*]

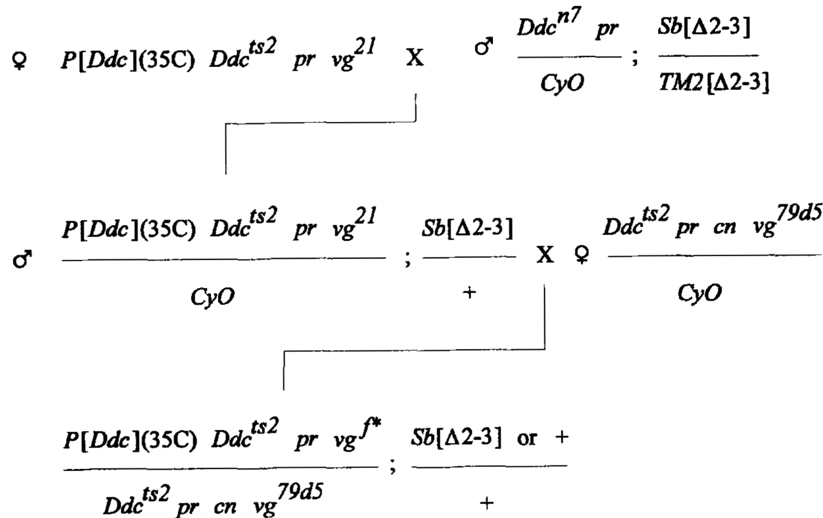


FIGURE 3.—Crossing scheme for targeting in *cis* using a more proximal donor. The crosses are designed to target *P*[*Ddc*], located at 35C, into vg^{21}. The progeny of the second cross are raised at 29°. Those progeny inheriting the putative targeted allele are denoted as vg^{f*}.

in vg^{28w} (HESLIP *et al.* 1992). Again, this polymorphism was present in all these DNA amplification products indicating that the 5′ *P* element in these lines was derived from the donor elements and not the original *P* element of vg^{21}. The amplification products of vg^{f49}, vg^{x336} and vg^{x347} using primers #1 and #4 were also sequenced. The polymorphism is maintained in the 5′ *P* element sequences of these lines as would be expected if the 5′ *P* element sequence of the donor has replaced the 3′ *P* element sequence of vg^{21}.

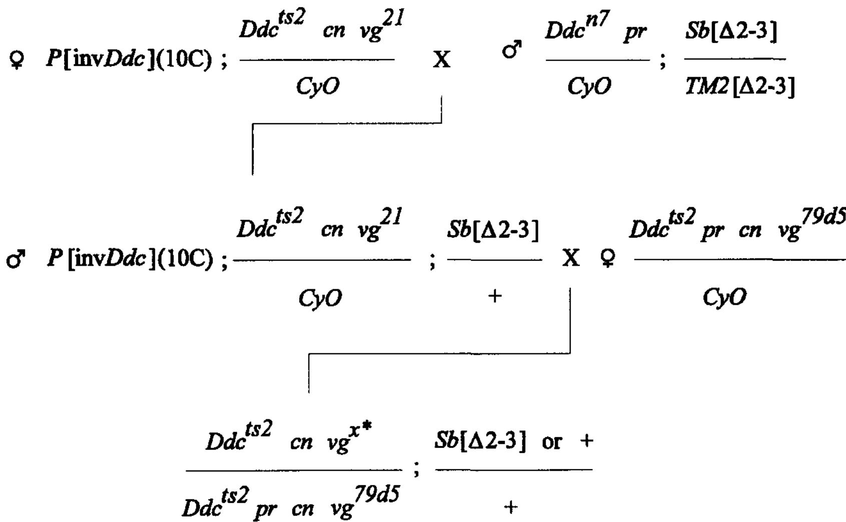
In addition to sequencing the DNA amplification products mentioned above, the opposite junction of each targeted insertion was also sequenced from appropriate DNA amplification products. In all cases, both junctions of the targeted insertions revealed precise replacement of the original vg^{21} *P* element with the new insertion leaving the 8-bp duplication flanking the insertion of vg^{21} and the rest of the *vg* sequence intact.

Southern analysis: All established lines containing targeted *vg* alleles were subjected to Southern analyses. In using DNA amplification to detect a targeted event, we test only for the insertion of those *Ddc* sequences complementary to the *Ddc* primers at the *vg* locus. If a targeted event results in the deletion of either the *vg* primer sequence or the appropriate *Ddc* sequence, then an amplification product would not be observed. For this reason, any lines that had a strong homozygous phenotype but did not yield an amplification product were also analyzed by Southern blots (data not shown).

During the maintenance of line x347 and from an initial Southern blot (data not shown), it was apparent that more than one targeted *vg* allele was segregating from the stock. This is probably because the founding parent of line x347 inherited a *Sb*[Δ2-3]-containing third chromosome which could have caused the original targeted transposition to be followed by a rearrangement. To eliminate the heterogeneity in this stock, several males from line x347 that were heterozygous for *CyO* were used to make sublines. Based on a subsequent Southern and DNA amplifications (data not shown), two sublines, x347.2 and x347.11, representing the two targeted *vg* alleles in line x347, were established and used for further analysis.

Lines a207, f38, x347.2 and x347.11 were included on the Southern blot shown in Figure 5. Strains used as controls include those containing the donor and target from the first cross of each targeting experiment (Figures 2, 3 and 4a) and the wild-type strain *Canton-S*. The genomic DNA samples were digested with *Eco*RI. The blot was hybridized with the 0.7-kb *Eco*RI fragment from vg^+ shown in Figure 2a (hereafter referred to as the vg^+ probe). The resulting autoradiograph is shown in Figure 5a, and we account for each band as follows. The 0.7-kb band seen in the first lane is due to the vg^+ allele present in the *Canton-S* control strain. The same band is present in the all other lanes, except lane 4 (a strain that does not contain *CyO*), due to the vg^+ allele present on the *CyO* chromosome. The control lanes 2, 4 and 6 show a 1.3-kb band due to the insertion of the 687-bp *P* element at *vg* as illustrated in Figure 2b. If a complete *P*[*Ddc*] is targeted to *vg* in either experiments a or f, regardless of the orientation of the insert, we would expect to see a 1.1-kb band and a 3.5-kb band. This is because the site of insertion at *vg* is centered between *Eco*RI sites (Figure 2, b and c). Lane 3 (a207) shows a 1.1-kb band and another band, 3.8-kb, that is slightly larger than expected. Lane 5 (f38) shows the 1.1-kb band and again a much larger band than expected, 5.3-kb. The unexpected bands in these two strains suggest an internal rearrangement of targeted *P*[*Ddc*] sequences such that larger *Eco*RI fragments were generated than predicted. If a complete *P*[inv*Ddc*] is present at *vg* from experiment x, we expect to see a 3.5-kb band and a 5.0-kb band in lane 7 or 8 due to the *Eco*RI sites in *P*[inv*Ddc*] (Figure 2d). Lane 7 shows a 1.8-kb band and lane 8 shows the expected 3.5- and 5.0-kb bands. The vg^+ probe, therefore, reveals that all four lines, a207, f38, x347.2 and x347.11 contain insertions at *vg*.

a.



b.

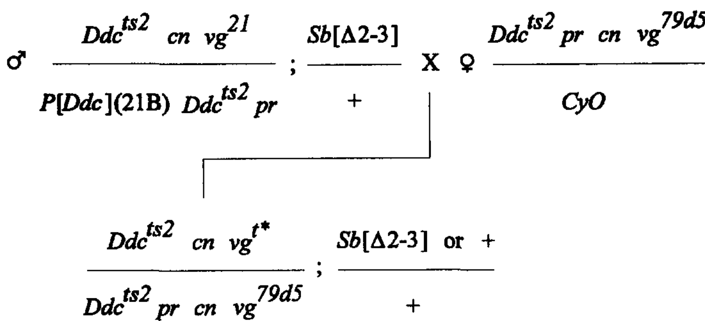


FIGURE 4.—Crossing schemes for targeting in *trans*. (a) These crosses are designed to target *P*[inv*Ddc*], located at 10C on the *X* chromosome, into vg^{21}. The progeny of the second cross are raised at 29°. The progeny which inherit vg^{x*}, the putative targeted allele, are detected by their more extreme mutant *vg* phenotype and used to establish stocks. The *P*[inv*Ddc*] (10C) containing *X* chromosome is marked with *y*. (b) The last cross from Figure 2a of HESLIP *et al.* (1992) is shown. The crosses are designed to target *P*[*Ddc*] (21B) from a *trans* position on the non-inverted homologous second chromosome into vg^{21}. This occurs in the germline of the male parents shown. The progeny of this cross are raised at 29°. The progeny inheriting a potentially targeted allele, vg^{t*}, are used to establish stocks.

To demonstrate that the insertions at *vg* contained *Ddc* sequences in these lines, the blot was stripped and probed sequentially with restriction fragments from *Ddc* (Figure 2c). Any genomic *Eco*RI restriction fragment that contains both *Ddc* and *vg* sequences will hybridize to both the vg^+ probe and the respective *Ddc* probe. This will result in a comigration of bands on autoradiographs taken from the blot after hybridization of these probes. The first *Ddc* probe used was the 5′ 2.5-kb *Pst*I/*Eco*RI fragment (hereafter referred to as the 5′ *Ddc* probe) shown in Figure 2c. The resulting autoradiograph is shown in Figure 5b. A 7-kb band is seen in all lanes from the endogenous *Ddc* locus. Lanes 2 and 3 also show a band slightly larger than 7-kb due to the donor *P*[*Ddc*] at 21B. Lanes 4 and 5 show a 5.4-kb band due to the donor *P*[*Ddc*] at 35C. In lane 3 (line a207) a 3.8-kb band is present which is the same size as that observed when the vg^+ probe was hybridized to the blot. Lane 5 (line f38) shows a band of 5.3-kb that comigrates with the band seen when using the vg^+ probe. Lane 6 (*P*[inv*Ddc*] (10C); Ddc^{ts2} *cn* vg^{21}/CyO) contains a 4.5-kb band due to the *P*[inv*Ddc*] on the *X* chromosome of this control strain. The original Southern performed with line 347 also showed the 4.5-kb band in the heterogeneous stock demonstrating that the donor was still present in this line also (data not shown). Lane 7 (line x347.2) showed only the 7-kb band from the *Ddc* locus. Lane 8 (line x347.11) shows a 3.5-kb band that was also seen when using the vg^+ probe. The results employing the 5′ *Ddc* probe indicate the presence of 5′ *Ddc* sequences at *vg* in lines a207, f38 and x347.11.

The blot was stripped again and re-probed (data not shown) with the 4.2-kb *Eco*RI/*Pst*I *Ddc* fragment (hereafter known as the 3′ *Ddc* probe) shown in Figure 2c. The only co-migration observed was a 5.0-kb *Eco*RI band in lane 8 (line x347.11) which also hybridized to the vg^+ probe. This indicates the presence of 3′ *Ddc* sequences at *vg* in line x347.11. The combination of *vg* and *Ddc* probes suggests that line x347.2 does not contain *Ddc* sequences at *vg* but that a small insertion remains probably consisting of the *P* element portion of *P*[inv*Ddc*].

The restriction maps of *P*[*Ddc*], *P*[inv*Ddc*], and *vg*, and the Southern analysis above, led us to predict that only line x347.11 had a complete *Ddc* gene inserted at *vg*. In support of this, measurements of DDC activity confirmed that the *Ddc* transgene in $vg^{x347.11}$ is functioning at a nearly normal level (data not shown). Further Southern analysis of lines a207, f38 and x347.11 was

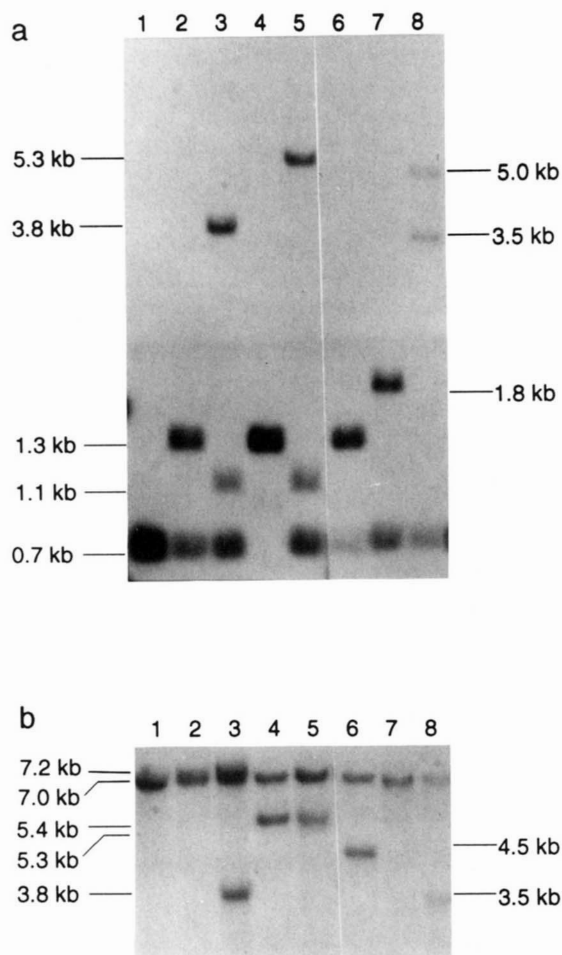


FIGURE 5.—Southern blot analysis. Genomic DNA was digested with *Eco*RI. The sizes of informative bands discussed in the text are indicated beside each autoradiograph. The lanes in (a) and (b) contain the following strains: lane 1, *Canton-S*; lane 2, *P*[*Ddc*](21B) *pr* Ddc^{ts2} vg^{21}/CyO; lane 3, *P*[*Ddc*](21B) *pr* Ddc^{ts2} vg^{a207}/CyO; lane 4, *P*[*Ddc*](35C) *pr* Ddc^{ts2} vg^{21}; lane 5, *P*[*Ddc*](35C) *pr* Ddc^{ts2} vg^{f38}/CyO; lane 6, *P*[inv*Ddc*](10C); Ddc^{ts2} *cn* vg^{21}/CyO; lane 7, Ddc^{ts2} *cn* $vg^{x347.2}/CyO$; lane 8, Ddc^{ts2} *cn* $vg^{x347.11}/CyO$. (a) Autoradiograph using the vg^{+} probe (Figure 2a). (b) The blot from (a) was stripped and rehybridized with the 5′ *Ddc* probe (Figure 2c). The double bands present in lanes 2,3, and 5 (discussed in *Southern analysis*) were confirmed using another Southern blot containing these strains in which the electrophoresis was run for a longer period of time (data not shown).

carried out to examine the size of the targeted *Ddc*-containing *P* elements (data not shown). Genomic DNA samples from these lines were digested with *Pst*I which liberates a 7.6-kb fragment from both the *Ddc* locus and any complete *P*[*Ddc*] or *P*[inv*Ddc*]. The Southern blot was hybridized sequentially with the *Ddc* probes mentioned above and the central 0.9-kb *Eco*RI *Ddc* fragment shown in Figure 2c. All three probes hybridized to a single 7.6-kb band in line x347.11 confirming the integrity of *P*[*Ddc*] in this line. Line a207 revealed the same 7.6-kb band and a 4.1-kb band when using the 5′ *Ddc* probe, the 3′ *Ddc* probe, and the central 0.9-kb *Eco*RI *Ddc* probe indicating a large internal deletion of the *P*[*Ddc*] in vg^{a207}. Line f38 showed the 7.6-kb band with all three *Ddc* probes but showed a smaller 3.9-kb band with only the 5′ and 3′ *Ddc* probes, not the central 0.9-kb *Eco*RI *Ddc* probe. This indicates an internal deletion of the *P*[*Ddc*] at vg^{f38} that includes the central 0.9-kb *Eco*RI fragment of *Ddc*. These observations confirm that x347.11 is the only targeted line with a complete *Ddc* gene at *vg* and that lines a207 and f38 contain targeted *P*[*Ddc*] elements with internal deletions.

TABLE 2

Donor transpositions

Donor	Transpositions[a]	Flies tested
P[*Ddc*](21B)	1[b]	153
P[*Ddc*](35C)	11[b]	72
P[inv*Ddc*](10C)	12[c]	95

[a] Calculation of the weighted and unweighted variances, V_w and V_u, respectively (ENGELS 1979), showed that V_w was less than V_u. According to ENGELS (1979), therefore, the weighted mean is appropriate for calculating the expected values for transpositions to new chromosomes and thus was used in a chi-square test. The chi-square test gave a $\chi^2 = 18.78$ ($P < 0.001$) indicating that the differences in transpositions between these three experiments are significant.

[b] Using primers #3 and #5.

[c] Using primers #2 and #5.

Mobility of donor *P* elements: We wished to address the question of whether a transposition intermediate or a genomic copy of the donor *P* element is used in the targeted transposition. If transposition intermediates are used, then mobility of the donors should parallel the rate of targeted transposition. Mobility of the donors was estimated using a transposition assay similar to that of ENGELS *et al.* (1990). Single fly DNA amplification was performed on segregants of experiments a, f and x that did not inherit the original donor *P* element containing chromosome. These were Ddc^{ts2} *pr cn* vg^{79d5}/CyO progeny of the second cross from experiments a and f and male progeny from the second cross of experiment x (Figures 1, 3 and 4a). Appropriate individuals were tested for the presence of *P*[*Ddc*] (experiments a and f) using primers #3 and #5 (Figure 2c) or for the presence of *P*[inv*Ddc*] (experiment x) using primers #2 and #5 (Figure 2c). If a product is observed then a transposition of the donor element to another chromosome must have occurred. The results of all mobility experiments are shown in Table 2. The ability of *P*[*Ddc*](21B) to move to another chromosome was significantly less than that for *P*[inv*Ddc*](10C) or *P*[*Ddc*](35C) (see footnote *a* of Table 2). These results suggest that a transposition intermediate of the donor *P* element is not used for targeted transposition because there was a large difference between the mobility of *P*[*Ddc*](21B) and the other two donors while the rates of targeted transposition were similar among all three.

DISCUSSION

The usefulness of *P* element transformation in studying the regulation of gene expression is limited by po-

sition effects (SCHOLNICK *et al.* 1983; SPRADLING and RUBIN 1983; CHEN and HODGETTS 1987). If a modified gene varies only slightly in its expression compared to the wild-type version, then it is difficult to ascribe the observed expression of this transgene to modifications made in regulatory sequences. There are a number of ways to circumvent the problem of position effects in studies of gene regulation. First, the use of insulator sequences to surround a gene within *P* element transformation vectors may shield it from position effects (KELLUM and SCHEDL 1991, 1992; ROSEMAN *et al.* 1993). However, in the absence of any quantitative analysis to date, it is not clear whether these potential chromatin domain boundary sequences are able to completely shield genes from position effects. Another method that potentially circumvents position effect difficulties is targeted gene replacement which can be used to alter sequences of interest at the endogenous locus (GLOOR *et al.* 1991; NASSIF *et al.* 1994). This method is useful for genes with a visible or easily detected mutant phenotype and for which a *P* element insertion near the sequence to be changed is available. Finally, there is targeted transposition, by which *P* elements constructs can be recovered at a constant position in the genome. This subjects the constructs to the same position effect and is ideal for studying the regulation of genes for which neither a *P* element induced allele nor easily observed phenotype is available. The unique features of targeted transposition make this method complementary to existing techniques for studying the regulation of gene expression. Of the three targeted alleles for which lines were established, Southern analysis demonstrated that two contained internal deletions of the *Ddc* gene. We note that the only targeted allele recovered from previous experiments contained a complete *P*[*Ddc*] (HESLIP *et al.* 1992). We suggest these deletions do not reflect a constraint on the amount of DNA that can be targeted to *vg*, because a 11.9-kb enhancer trap has also been targeted to the locus (STAVELEY *et al.* 1994). Whether or not the deletions arise because of the internal sequence organization of the *Ddc* gene remains to be determined.

We have examined a number variables for their effect on the frequency of targeted transposition. Experiments a, f and x demonstrate that the rate of targeted transposition was similar using donor elements in a *cis* configuration or in a *trans* configuration on a non-homologous chromosome and when using proximal *vs.* distal donor elements in *cis*. The differences in targeted transposition rates were tested by chi-square analysis. The result ($P \geq 0.85$) was not very informative because of the low number of targeted alleles obtained, but suggests that differences in the frequencies of targeted transposition were not significant. The average rate was approximately 3 events per 7×10^3 flies scored. The screening of all flies with increased *vg* phenotypes as well as a significant number of borderline mutants using DNA amplification ensured that no targeting events were overlooked. However, our results showed that this rigorous screening was unnecessary since all nine of the targeted events reported in Table 1 produced a strong mutant phenotype.

In experiment x, the targeting was carried out in a vg^{21}/CyO heterozygote. In contrast, the t experiment was performed in a vg^{21} heterozygote that contained a non-inverted homologous second chromosome. Another difference between experiments x and t is the use of a donor in *trans* on a non-homologous chromosome relative to the target *vs.* a donor in *trans* on the homologous chromosome. One reason for the failure of the t experiment to yield a single targeted event could be due to the lack of a *CyO* chromosome. The status of the homologous chromosome is known to affect *P* element reversion at w^{hd} (ENGELS *et al.* 1990) and may also be important for targeted gene replacement. Since the *CyO* chromosome is rearranged by five inversions and the *vg* locus is included in one of these inversions (LINDSLEY and ZIMM 1992), it is possible that pairing of homologous *vg* sequences is disrupted in vg^{21}/CyO heterozygotes and that this may enhance the rate of targeted transposition. Another explanation for the failure of the t experiment is the location of the donor element. The presence of the donor in *trans* on the homologous chromosome may reduce or eliminate targeted transposition compared to donors in *trans* from non-homologous chromosomes. A combination of both variables may also have caused the lack of targeting in the t experiment. We are currently investigating the relationship between the structure of the homologous second chromosome used and the rate of both *P* element reversion and targeted transposition at *vg*.

The mechanism of targeted transposition is unknown. Replacement of the vg^{21} *P* element may depend on a transposition intermediate or a genomic copy of the donor element. If a transposition intermediate is used, the level of that intermediate, as measured by the rate of transposition to a different chromosome, should parallel the frequency of targeting. The mobility experiment, which was designed to measure the rate of transposition, demonstrated that *P*[*Ddc*](21B) is approximately one order of magnitude less mobile than either *P*[inv*Ddc*](10C) or *P*[*Ddc*](35C). Yet, the frequency of targeted transposition was similar between all three of these donors (Table 1). We interpret these results as an indication that targeted transposition does not use a transposition intermediate and may use a genomic copy of a donor element.

The targeted transpositions at the *yellow* locus were described as conversion events (GEYER *et al.* 1988). Several pieces of our data support the concept of a replicative, conversion-like mechanism for targeted transposition. First, the sequence polymorphism located at nucleotide #33 in the 5′ end of the donor *P* elements (HESLIP *et al.* 1992) was present in all targeted *vg* alleles

which contained the donor element in the same orientation as the original *P* element in vg^{21}. This indicates that targeted transposition resulted in a replacement of the 5′ *P* element sequences of vg^{21} with 5′ *P* element sequences from the donor. Second, complete donor elements were still present at their original locations in lines a207, f38 and x347. It is possible that homology between the vg^{21} *P* element and the *P* element sequences of *P*[*Ddc*] or *P*[inv*Ddc*] is used to guide the replacement of the target with a donor transposon. The fact that the *P* elements in vg^{f49}, vg^{x336} and $vg^{x347.11}$ are in the opposite orientation relative to the original vg^{21} *P* element indicates that no more than the 31-bp of inverted terminal repeat sequence is required for targeted transposition.

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