Topological Constraints on Transvection Between *white* Genes Within the Transposing Element *TE35B* in *Drosophila melanogaster*

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ABSTRACT

The transposable element *TE35B* carries two copies of the *white* (*w*) gene at 35B1.2 on the second chromosome. These *w* genes are suppressed in a *zeste-1* (z^1) mutant background in a synapsis-dependent manner. Single-copy derivatives of the original *TE35B* stock give red eyes when heterozygous, but zeste eyes when homozygous. *TE35B* derivatives carrying single, double or triple copies of *w* were crossed to generate flies carrying from two to five ectopic *w* genes. Within this range, z^1-mediated suppression is insensitive to copynumber and does not distinguish between *w* genes that are in *cis* or in *trans*. Suppression does not require the juxtaposition of even numbers of *w* genes, but is extremely sensitive to chromosomal topology. When arranged in a tight cluster, in triple-copy *TE* derivatives, *w* genes are nonsuppressible. Breakpoints falling within *TE35B* and separating two functional *w* genes act as partial suppressors of z^1. Similarly, breakpoints immediately proximal or distal to both *w* genes give partial suppression. This transvection-dependent downregulation of *w* genes may result from mis-activation of the *X*-chromosome dosage compensation mechanism.

TE35B is one of a family of transposing elements described by ISING and coworkers (see ISING and BLOCK 1981). It is inserted on chromosome arm 2L at 35B1.2 within the *no-ocelli* (*noc*; 2-50) gene (CHIA *et al.* 1985). The genetic properties of *TE35B* have been described by GUBB *et al.* (1985, 1986) and its molecular properties by LOVERING *et al.* (1991).

ISING's family of transposing elements are composite structures carrying the *white* (*w*) and *roughest* (*rst*) loci from the *X* chromosome flanked by *Foldback* (*FB*) elements (POTTER *et al.* 1980; PARO *et al.* 1983). *TE35B(Z)* is a tandem duplication of ISING's original *TE*. It carries two functional copies of w^+ and rst^+ by several criteria including that z^1 *w*; *TE35B(Z)*/+ flies are phenotypically zeste and that *in situ* hybridization to *TE35B(Z)* shows two sites of homology to a *w* probe (GUBB *et al.* 1985). The original *TE* of ISING's family (*TE48F*) was derived from the *Basc* chromosome and carried a *white-apricot* (w^a) allele (ISING and BLOCK 1981). The w^a mutation is associated with a *copia* element (GEHRING and PARO 1980; BINGHAM and JUDD 1981). Many of the derivatives of *TE48F*, including *TE35B(Z)*, carry partial revertants of the w^a allele, which give a red-eyed phenotype. These revertants retain *copia* homology (GEHRING and PARO 1980) and express about a third the eye-pigment of a wild-type *w* allele. The *w* genes on *TE35B* continue to dosage compensate as though they remained *X*-linked (GUBB *et al.* 1986), which results in a darker eye color in *TE35B* males than females. The *TE*-borne w^a revertant is designated w^+ in the remainder of this article although it is less active than a true wild-type allele.

The z^1 mutation of *D. melanogaster* results in females with lemon-yellow eyes. The males retain the wild type, red eye color (GANS-DAVID 1949). GANS (1953) showed that the expression of z^1 required two copies of w^+. As both *zeste* and *white* are *X*-linked genes, a male, being hemizygous for z^1 and w^+, will not express *zeste*. A female, being homozygous for z^1 and w^+, will express *zeste*. Males that are hemizygous for z^1, but carry a tandem duplication of w^+, express *zeste*. Conversely, females that are homozygous for z^1, but heterozygous for a deletion of *w*, are red-eyed. In the interaction between these two genes, z^1 can be regarded as a suppressor of w^+ (JACK and JUDD 1979). The suppression occurs at the transcriptional level with reduced quantities of *w* transcript being found in z^1 w^+ flies (PIRROTTA and BROCKL 1984). This suppression is a neomorphic function of the ZESTE-1 protein; the wild-type ZESTE protein acts as a transcriptional activator (PIRROTTA *et al.* 1987; PIRROTTA 1991).

With respect to the *zeste-white* interaction, there are two classes of *white* mutation. One is equivalent to a deletion and acts as a dominant suppressor of z^1. The other behaves as $white^+$ and does not suppress z^1. GREEN (1959) showed that only the proximal region of *white* is required for the interaction with z^1. Mutations in the distal region remain suppressed by z^1. The region required for z^1 suppression of w^+ is contained within a 771-bp sequence ~1.25 kb 5′ to the site of initiation of transcription of *w* (HAZELRIGG *et al.* 1984; PIRROTTA *et al.* 1985).

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There is, however, an additional requirement for suppression to occur; the two copies of w^+ must be close to each other. GELBART (1971) used transpositions of w^+ to construct genotypes carrying one copy of w^+ on the *X* and one on an autosome. Flies of these genotypes were not phenotypically zeste. When the autosomal copies of w^+ were homozygous, in z^1 w^{11E4}; *Dp(1;A)*w^+/*Dp(1;A)*w^+ flies, the zeste eye phenotype was restored. Thus, the w^+ genes must be adjacent, but their spatial relationship to the site of the *zeste* gene is unimportant.

The zeste phenotype shown by a tandem duplication of the proximal region of *w*, in z^1 *Dp(1;1)*w^{+R} males, is indistinguishable from that of homozygous z^1 w^+ females (GREEN 1959). Similarly, the tandem duplication of *w*, in z^1 w^{11E4}; *TE35B(Z)*/+ flies, gives a zeste phenotype in both males and females (GUBB *et al.* 1985). It is important to realize that the *w-w* interaction required for the suppression of *w* gene activity in a z^1 background can occur either in *cis* or *trans*. As pointed out by JACK and JUDD (1979), the interaction between *w* genes resembles transvection as described by LEWIS (1954) for the *bithorax* complex.

In this article, the *cis* and *trans* interactions between copies of the *w* gene are shown to reflect similar, if not identical, phenomena. The proximity requirement for the *w-w* interaction appears to be extremely stringent and can only occur when the chromatin domains immediately surrounding the *w* gene are synapsed.

The "*zeste*" form of *TE35B, TE35B(Z)* is unstable. It gives "spontaneous red" (*SR*) forms with a frequency of about one in 2600 flies (GUBB *et al.* 1986). Most of these red-eyed derivatives result from loss of half of *TE35B(Z),* leaving a single w^+ gene. They are cytologically smaller than *TE35B(Z),* being visible as two- to three-band insertions, rather than the five to six bands of the original form. As would be expected, the single-copy, *TE35B(Z)SR* derivatives give a zeste phenotype when homozygous, in z^1 w^{11E4}; *TE35B(Z)SR/ TE35B(Z)SR* flies. The zeste phenotype is also expressed when single-copy, *TE35B(Z)SR* derivatives are heterozygous with the original *TE35B(Z)* chromosome or when the original *TE35B(Z)* chromosome is homozygous. Thus, two, three or four adjacent copies of *w* can be suppressed in a z^1 background. Surprisingly, the zeste eye color does not darken with increasing number of w^+ genes and is not sexually dimorphic, so that males with four w^+ genes have eyes that are no lighter than females with two w^+ genes.

In addition to the half-loss *TE35B(Z)SR* derivatives, a rarer class of *TE35B(Z)SR* chromosomes (one in 14,000, GUBB *et al.* 1986) was recovered that are not *zeste*-suppressible. Non-suppressible *SR*s remain red-eyed when heterozygous with *TE35B(Z)* or *TE35B(Z)SR100,* indicating that one, or more, copies of w^+ fail to transvect. Non-suppressible stocks can result from transposition of half of the *TE* to a novel site as occurred in *SR103* (GUBB *et al.* 1986), which corresponds to z^1 w^{11E4}; *TE31AB(R)* + *TE35B(Z).* Two other non-suppressible *SR* stocks from this earlier study, *SR36* and *SR23,* are shown here to carry a compact array of three w^+ genes. These w^+ genes remain functional by both genetic and molecular criteria, but refractory to suppression in a z^1 background unless rearranged by a chromosomal aberration.

Localized topological constraints that affect the zeste phenotype of *TE35B(Z)* can also be caused by breakpoints close to 35B on the homologous second chromosome (GUBB *et al.* 1990). The purpose of this paper is to characterize both spontaneous and gamma ray-induced changes on the *TE35B(Z)* chromosome that suppress transvection between intact w^+ genes. These results have implications for the basis of transvection at *bithorax* (LEWIS 1954), *decapentaplegic* (GELBART 1982) and *eyes absent* (LEISERSON *et al.* 1994) where much longer-range disruption of synapsis occurs.

MATERIALS AND METHODS

Nomenclature: The original, tandem-duplicated form of the *TE* was recovered by ISING and coworkers and designated *TE146* (ISING and BLOCK 1981). Following the rules suggested by LINDSLEY and ZIMM (1992) this nomenclature has been changed to *TE35B* to indicate the cytological insertion site of the *TE* at 35B1.2. The single-copy *TE* named *TE36* by ISING and BLOCK (1981), which inserts at 35B10-C1, becomes *TE35BC* on this nomenclature. *TE35BC(R)* is a single-copy w^+ rst^+ *TE* associated with a lethal mutation at the *ck* locus (GUBB *et al.* 1984), ~10 complementation groups proximal to *TE35B.*

To distinguish the different derivatives of *TE35B,* the original double-copy form of the *TE* is designated *TE35B(Z)* reflecting the zeste eye color of z^1 w^{11E4}; *TE35B(Z)*/+ flies. Derivative chromosomes are prefixed *G,* if gamma ray-induced, or *S,* if spontaneous; followed by *R* for red-eyed, *V* if variegating and *Z* for zeste-eyed. This format allows the progenitor chromosome to be indicated within parentheses as in *TE35B(Z) GR1* for the first gamma ray-induced red-eyed derivative of the original zeste form of *TE35B.* Subsequent changes can be included within parentheses, so that *TE35B(Z:SR36)SZ4* is the fourth spontaneous zeste-eyed derivative of *SR36,* which was the 36th spontaneous red-eyed derivative of the original form (GUBB *et al.* 1986) although in most contexts this designation would be shortened to *SZ4.*

The conventions for describing autosynaptic stocks follow CRAYMER (1981) as modified in GUBB *et al.* (1988). The description of chromosomal aberrations, in particular the translocation segregant elements, follows FlyBase nomenclature rules.

Cytology and *in situ* hybridization: Temporary propionic-orcein-carmine-stained preparations of salivary gland polytene chromosomes were prepared from larvae grown on yeast-glucose medium and interpreted using the revised chromosome maps of C. B. and P. N. BRIDGES (see LEFEVRE 1976). *In situ* hybridization was done as described GUBB *et al.* (1986) using nick-translated biotinylated probes detected by the horseradish peroxidase catalyzed polymerization of 3,3′-diaminobenzidine.

Probes: The probe used for the *w* gene is the 10.78-kb *Kpn*I-*Eco*RI fragment, subcloned from the *km2.1* phage insert (LEVIS *et al.* 1982) by K. MOSES. For Southern hybridization, a *w*

cDNA, kindly provided by S. POTTER, was used. Probes to the proximal and distal boundaries of *TE35B* were as described by LOVERING *et al.* (1991), while probes to the second chromosomal sequences adjacent to the *TE* insertion site were as described by DAVIS *et al.* (1990).

Southern hybridization: Standard techniques of DNA preparation and digestion were used as described in SAMBROOK *et al.* (1989).

Pigment assays: Pigments were extracted from the heads of 6-day-old flies at room temperature. Five heads were soaked overnight in 0.8 ml of acid-ethanol (30% ethanol acidified to pH 2 with HCl) and then were homogenized and centrifuged to remove debris. Absorbance of the extract was read spectrophotometrically at 480 nm corrected against an acid-ethanol blank. This procedure was modified from CLANCY (1942). A minimum of five independent assays of each sex of each genotype was made. To correct for the size difference between male and female flies, heads of wild-type (Canton-S) flies were weighed. The ratio was 1:1.3 (100 heads of Canton S males weigh 0.90 mg compared to 1.17 mg for 100 female heads). To compensate for this difference, male values in the pigment assays have been increased by a factor of 1.3.

To check that pigment extraction and densitometry were linear over the measured range, between one and 40 heads were extracted in 0.8 ml of acid-ethanol. The calibration curve was linear up to 30 heads, for either sex, but began to plateau between 30 and 40 heads. In the first screen, the *TE35B(Z)* chromosome carried the recessive eye color mutations *purple* (*pr*) and *cinnabar* (*cn*). Neither of these mutations have a measurable effect, when heterozygous, on the levels of extractable eye pigment under the assay conditions used. To avoid the potential problem caused by these recessive mutations, all pigment assays were made using flies carrying the *TE* chromosome heterozygous with a wild-type second chromosome.

Stock design: All stocks used in the screens to recover aberrations on the *TE35B(Z)* chromosome carried the z^1 mutation and a deletion for the *white* locus, w^{11E4} (ZACHAR and BINGHAM 1982). Since *TE35B* is homozygous viable, spontaneous loss of half of *TE35B(Z)* can give *TE35B(Z)SR* flies that remain phenotypically zeste when heterozygous with the original *TE35B(Z)* chromosome (GUBB *et al.* 1986), but will give red-eyed flies when out-crossed. To avoid this problem, heterozygous *TE* stocks were constructed using the *In(2LR)O, Cy* dp^{lvl} *pr* cn^2 *(CyO)* balancer chromosome, and a lethal mutation, *pawn (l(2)pwn),* was recombined onto the *TE* chromosome. This has the disadvantage that the derived *GR* chromosomes are lethal with each other, unless *l(2)pwn* is removed by recombination. For this reason a homozygous viable *b TE35B* chromosome was used in subsequent screens. The viable recessive markers and the *CyO* chromosome are described in LINDSLEY and ZIMM (1992).

Crosses: One- to 4-day-old males were irradiated with 4.5 kR gamma rays from a ^{60}Co source and mated to virgin females in 200 ml bottles. One hundred fifty pairs of flies were used per bottle. Parental flies were transferred every 3 days to bottles that were coded so that clusters of exceptional progeny from the same parents could be identified. Progeny from each bottle were scored up to the 18th day after setting up. Cultures were grown at 25° on yeast-glucose medium. The eye-color phenotype of parental flies was carefully checked before setting up crosses and any nonzeste flies were discarded.

In the first screen, irradiated z^1 w^{11E4}; *al dp b TE35B(Z) pr l(2)pwn cn/CyO* males were mated to females of the same genotype to recover stocks in the F_2. These stocks included *GR* mutations associated with *TE35B* and *Su(*z^1*)* mutations. Stocks segregating zeste-eyed progeny in succeeding generations were discarded as putative *Su(*z^1*)* mutations on nonhomologous chromosomes. Stocks carrying *Su(*z^1*)* mutations on the irradiated *CyO* chromosome were identified by crossing to z^1 w^{11E4}; *b TE35B(Z)/CyO, Cy* dp^{lvl} b^{81f1} *pr* cn^2. In the second screen, irradiated z^1 w^{11E4}; *b TE35B(Z)/CyO,* b^{81f1} males were crossed to z^1 w^{11E4}; +/+ females. This had the advantage that any *Su(*z^1*)* mutations on the irradiated *CyO* chromosome would segregate from the irradiated *b TE35B(Z)* chromosome and be discarded among the white Curly progeny. The irradiated second chromosome was recovered by mating putative z^1 w^{11E4}; *b TE35B(Z)GR/+* flies to z^1 w^{11E4}; *Gla/CyO,* b^{81f1} and selecting Cy b progeny with the modified eye color to establish stocks.

Separation and mapping of aberration breakpoints: In 12 cases the cytological breakpoints of the *GR* aberrations (Table 1) were similar enough to those of preexisting aberrations (Table 2) to allow the proximal and distal elements to be separated genetically. This allowed the breakpoints to be mapped, both with respect to the second chromosomal loci in 35B and the w^+ and rst^+ genes carried on the *TE.*

The proximal and distal elements of *GR* translocations were separated as the segregants carrying the second left telomere, *Ts(2Lt),* and second right telomere, *Ts(2Rt),* respectively. The left and right elements of paracentric inversions were separated following recombination with similar inversions. Pericentric inversion breakpoints were separated using CRAYMER's (1981) autosynaptic method. For this purpose, the autosynaptic stocks, z^1 w^{11E4}; *LS(2)Sco*^{rv1}, *net//DS(2)Sco*^{rv1}, *bw sp* and z^1 w^{11E4}; *LS(2)noc*^{4}, *b//DS(2)noc*^{4}, *cn sp* were constructed carrying homozygous recessive markers on both chromosomal arms.

Where possible, the aberrations used were chosen so that both elements of novel aberrations were recovered as synthetic deletions around the *TE35B* insertion site. These deletions were then mapped genetically to confirm the identity of the recovered products and the precise site of the breakpoint using deletions in the 35B region (ROOTE *et al.* 1996) and the mutations listed in Table 2.

RESULTS

Transvection between two, three and four w^2 genes: The original form of *TE35B* carries a tandem repeat of w^+ and rst^+ and gives a zeste eye color, indistinguishable from that of female z^1 w^+ flies. *TE35B(Z)* is visible as a five- to six-band insertion when heterozygous in salivary gland polytene chromosomes (GUBB *et al.* 1985). *In situ* hybridization with a *w* probe gives either a V-shaped band of staining or parallel double-bands. We interpret these two patterns as corresponding to side-on and face-on views of the *TE35B(Z)* chromosome when synapsed tightly with a wild-type homologue (Figure 1B). In homozygous z^1 w^{11E4}; *TE35B(Z)/TE35B(Z)* flies the eye color remains zeste. Spontaneous loss of either the proximal or distal half of the original form gives single-copy *SR* derivatives (GUBB *et al.* 1986; LOVERING *et al.* 1991). The single-copy *SR* derivatives give zeste eyes either when homozygous, or when heterozygous with the original double copy form. Thus, two, three or four adjacent copies of w^+ are capable of transvecting in the following genotypes: z^1 w^{11E4}; *TE35B(Z)SR22/TE35B (Z)SR22,* z^1 w^{11E4}; *TE35B(Z)/+,* z^1 w^{11E4}; *TE35B(Z)SR22/TE35B(Z)* and z^1 w^{11E4}; *TE35B(Z)/TE35B(Z).*

The single-copy form *TE35BC(R)* also gives a zeste

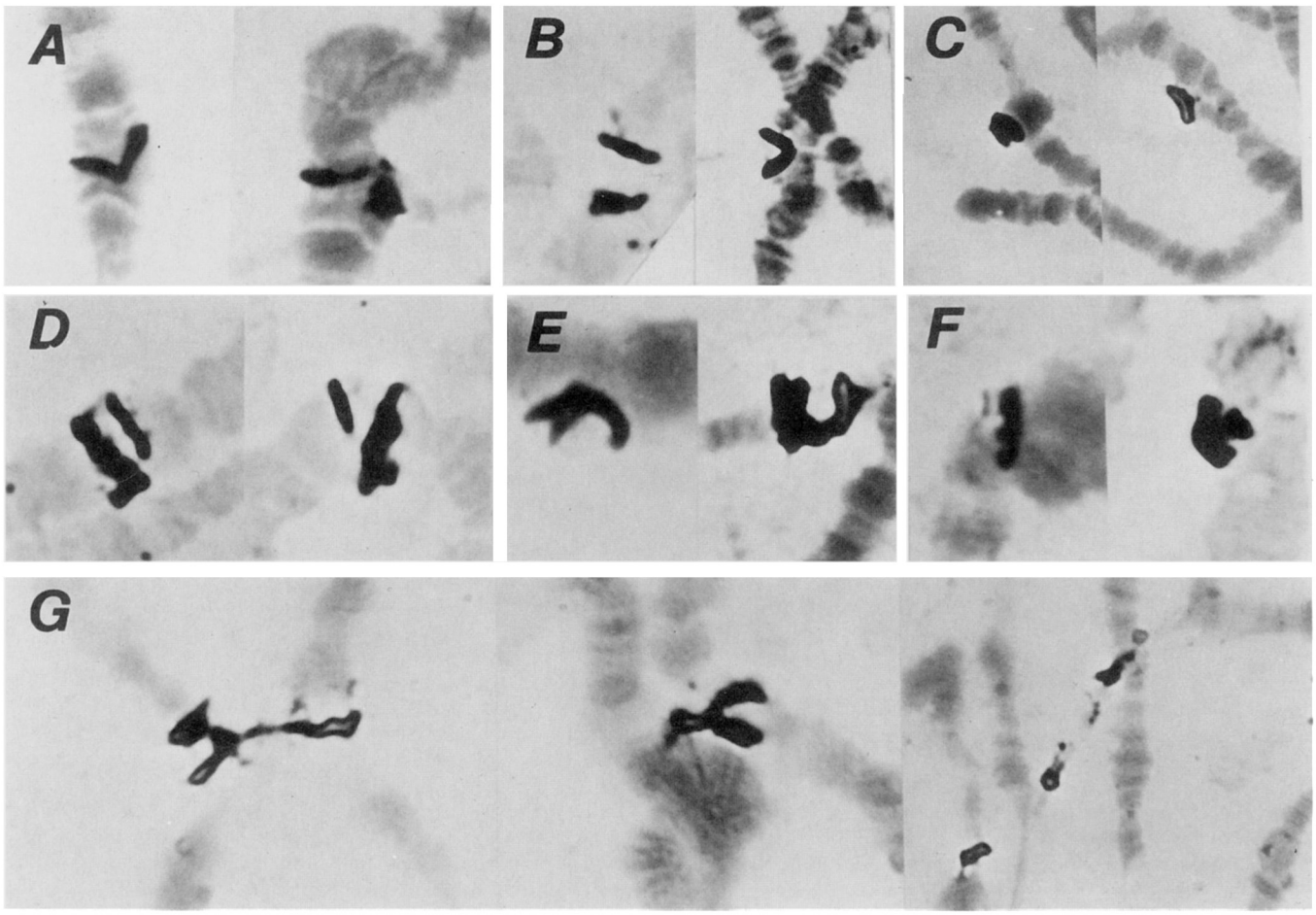

FIGURE 1.—Chromosomal *in situ*'s with a *w* probe to different spontaneous *TE* derivatives. (A) Heterozygous displaced single-copy derivatives *TE35B(SR100)/TE35BC(R)*. The region between the two insertion sites (35B1.2 and 35B10-C1) is frequently distorted with the two sites of homology in close apposition. Eye phenotype is zeste, in z^1 background. (B) Tandem double-copy form *TE35B(Z)/+*, front and side views, w^+ rst^+ w^+ rst^+. (C) Reversed double-copy form *SZ1/+*, rst^+ w^+ w^+ rst^+. The *TE* pairs as a hairpin-loop with the sites of homology fused as a composite band. (D) Tandem/reversed heterozygote *TE35B(Z)/SZ1*, w^+ rst^+ w^+ rst^+ /rst^+ w^+ w^+ rst^+. The hybridization pattern is variable depending on the angle of view but, often appears as three compact and one single band. Eye phenotype, zeste. (E) Triple-copy form *SR36/+* showing a single, proximal site and a doublet distal site. Bands remain discrete implying that the structure corresponds to a tandem triplication 35B1|w^+ rst^+ w^+ rst^+ w^+ rst^+|35B2 with, perhaps, additional second chromosomal sequences duplicated between the medial and proximal copies of the *TE*. (F) The triple-copy form, *SR23/+*, appears as one large composite band; separate bands are never resolved. Possible structures resulting from insertion of a single TE within the original form are as follows: triple-hairpin (35B1|w^+ rst^+ rst^+ w^+ w^+ rst^+|35B2), hairpin-tandem (35B1|rst^+ w^+ w^+ rst^+ w^+ rst^+ |35B2), tandem-hairpin (35B1|w^+ rst^+ rst^+ w^+ w^+ rst^+ |35B2) or tandem triplication (35B1|w^+ rst^+ w^+ rst^+ w^+ rst^+|35B2). The triple-hairpin structure should allow stacking of the three *w* genes in register and might be expected to give a zeste phenotype. The tandem-hairpin and hairpin-tandem structures both require one of the *w* genes to pair in a tight loop and, therefore, remain nonzeste suppressible; although there is no cytological evidence for a hairpin structure. The tandem triplication form might be the most likely form as it would require three *w* genes to synapse as a tight double-loop. The bottom row (G) shows three examples of the zeste form *SZ4, In(2LR)TE35B-4, TE35B(Z:SR36)SZ4/+* that are stretched to different degrees. The spontaneous inversion appears to have separated the distal doublet of *SR36* to give a double-copy insertion at the 35B2|43A1 breakpoint leaving a single-copy at the 35B1|43A2 breakpoint. The double-copy insertion is probably tandem as it never pairs as a hair-pin loop. The most probable structure is therefore 35B1|w^+ rst^+ w^+ rst^+ |43A1—35B2|w^+ rst^+ rst^+ *43A2. In any case, the inversion loop allows the three sites of w* homology to be closely opposed.

eye in z^1 w^{11E4}; *TE35BC(R)/TE35B(Z)* and z^1 w^{11E4}; *TE35BC(R)/TE35B(Z)SR* flies. This suggests that the *w-w* interaction could be taking place across the 10, or so, complementation groups that separate the *TE* insertion sites, implying that the proximity requirement for suppression is rather loose. In general, *trans*-heterozygous combinations of *TE* chromosomes give zeste eyes provided the insertion sites are separated by less than one numbered chromosomal division (G. ISING and K. BLOCK, personal communication). An alternative to the loose proximity requirement is that *trans*-heterozygous *TE* combinations distort the intervening chromosomal regions allowing the w^+ genes to remain closely synapsed. This configuration has been observed in poly-

tene chromosomes for a number of pairs of *TE* insertion sites (G. ISING and K. BLOCK, personal communication) and is illustrated for heterozygous *TE35BC(R)/TE35B(Z)SR100* salivary glands in Figure 1A. For reasons that will become clear, we consider that ISING and BLOCK's observation is informative, that the proximity requirement is extremely stringent and that the configuration of polytene chromosomes gives a valid indication of chromosomal pairing in the pigment cells of the eye.

To test the proximity requirement further, heterozygous flies carrying the original and a reversed tandem form of *TE35B* were constructed. The reversed tandem form *TE35B(Z:SR100)SZ1* (GUBB *et al.* 1985; LOVERING *et al.* 1991) pairs as a hairpin loop in *TE35B-(Z:SR100)SZ1/+* larval salivary glands (Figure 1C) with the sites of homology to *w* adjacent to each other in the centre of the *TE*. When heterozygous with the original form, one of the four *w* genes would be in reversed orientation to the others, unless a very tight inversion loop were formed. The observed eye color of heterozygous z^1 w^{11E4}; *TE35B(Z:SR100)SZ1/TE35B(Z)* flies is zeste, but eyes have a crescent of red ommatidia along the posterior edge. In salivary gland chromosomes, this heterozygous four copy combination does not show either the hairpin or reversed loop configuration. Synapsis in the 35B region is relatively normal, but on a fine scale, the pattern of homology to a *w* probe is very variable. Chromosomes often show one thick band and one thin band of homology as if three w^+ genes were stacking in register and the fourth were unpaired (Figure 1D).

Two further *SR* derivatives that were known not to be *zeste* suppressible (GUBB *et al.* 1985) were examined by *in situ* hybridization. These derivatives, *SR23* and *SR36*, show a compact array of three sites of homology to *w* (Figure 1, E and F). The evidence that all three of these sites represent functional w^+ genes is, first, that these derivatives give a high pigment level (Table 6) and contain no restriction site polymorphisms within the *w* gene (data not presented). More critically, a spontaneous zeste derivative *TE35B(Z:SR36)SZ4* was recovered that gives the same pigment level as its progenitor (Table 6). The *SZ4* derivative carries an inversion with one breakpoint within the *TE* and the other in 43A1.2. This inversion carries a single red *TE* at its left breakpoint and a double-copy zeste *TE* on its right breakpoint. In salivary gland chromosomes, the inversion pairs as a loop allowing close proximity of the three sites of homology to *w* (Figure 1G). The inversion releases the local constraint on synapsis within the compact triple-copy *TE* by the criterion that z^1 w^{11E4}; *In(2LR)TE35B-4, TE35B(Z:SR36)SZ4/+* flies express a zeste phenotype indistinguishable from that of other *TE* combinations, including the heterozygous three-copy genotype z^1 w^{11E4}; *TE35B(Z)SR22/TE35B(Z)*. Although the pairing in triple-copy forms of the *TE* appears similar in polytene *in situ* preparations to that in *In(2LR)TE35B-4, TE35B(Z:SR36)SZ4*, the different phenotypes in a z^1 mutant background indicate that synapsis is suppressed in the pigment cells of the eye in *SR23* and *SR36* but not the inversion *TE35B-4* chromosome. In these examples, the alignment of *w* genes in polytene *in situ* preparations does not predict the degree of synapsis in eye pigment cell chromosomes, which are probably diploid.

As might be expected, the triple-copy inversion form, *In(2LR)TE35B-4, TE35B(Z:SR36)SZ4*, retains a zeste phenotype when heterozygous with single- or double-copy derivatives in z^1 w^{11E4}; *TE35B(Z)SR22/In(2LR)TE35B-4, TE35B(Z:SR36)SZ4* and z^1 w^{11E4}; *TE35B(Z)/In(2LR)TE35B-4, TE35B(Z:SR36)SZ4* flies. This final combination indicates that five copies of *w* in close proximity can be completely suppressed, although the compact triple-copy form remains red-eyed in the absence of the spontaneous *In(2LR)SZ4* inversion.

The structure of the single-, double- and triple-copy *TE* derivatives was confirmed on Southern blots with a probe to the proximal boundary of the *TE* insertion site (Figure 2). Note the increase in fusion fragments from two, in *TE35B(Z)*, to three, in *TE35B(Z)SR36*, and the further alteration in size of one of the fusion fragments in *TE35B(Z:SR36)SZ4*.

The recovery of induced non-zeste-eyed derivatives of the *TE35B(Z)* chromosome: Three classes of non-zeste-eyed fly were recovered after gamma-irradiation, *GW*, *GV* and *GR*. *GW* chromosomes result from either spontaneous or induced loss of the *TE* (GUBB *et al.* 1985, 1986) and were discarded in the present experiments. In the other two classes, nine *GV* and 72 *GR* stocks were established from 172 non-zeste-eyed progeny among 45,285 flies in three different screens. In all three screens, the frequency of recovery of non-zeste-eyed stocks was very high. In the first two screens, from heterozygous *TE35B(Z)* males, non-zeste-eyed stocks were established at a frequency of one in 349 and one in 557 irradiated chromosomes, respectively. In the third screen, stocks were recovered at a frequency of one in 240 chromosomes from irradiated homozygous z^1 w^{11E4}; *b TE35B(Z)/b TE35B(Z)* males.

Cytological examination of the *GV* chromosomes showed that each carried an aberration with one breakpoint at, or adjacent to, the *TE* and the other in heterochromatin: *T(Y;2)TE35B(Z)GV51, T(Y;2)TE35B(Z)-GV204, In(2L)TE35B(Z)GV63, In(2LR)TE35B(Z)GV50, T(2;3)TE35B(Z)GV202, T(2;3)TE35B(Z)GV209, T(2;3)-TE35B(Z)GV221, T(2;3)TE35B(Z)GV229* and *Tp(2;3)-TE35B(Z)GV203*. The variegation of w^+ in these chromosomes therefore results from heterochromatic position-effect variegation (HENIKOFF 1990). In a z^1 w^- genetic background, these *GV* chromosomes give a zeste eye with white sectors; while they give red eyes with white sectors in a z^+ w^- background. No flies with

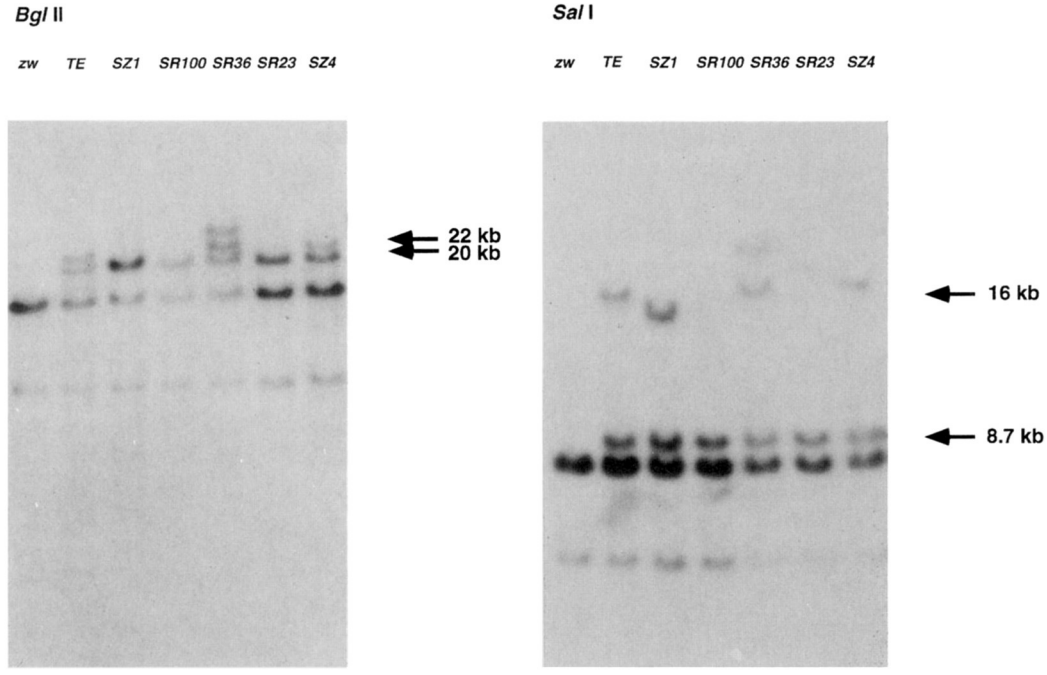

FIGURE 2.—Southern blots of *Bgl*II- and *Sal*I-digested DNA from *TE35B(Z)* and spontaneous derivative chromosomes. Filters were probed with the *rstSR*0.8 genomic fragment, which hybridizes to the proximal boundary of the *TE35B(Z)* insertion site and an internal *TE* fragment, Figure 5. With both *Bgl*II and *Sal*I, the common fragments, deriving from the (*z w*) *X* chromosome, are smaller than the *TE35B* fusion fragments; compare *z w* and *TE* tracks. In the *TE35B(Z)* derivative chromosomes, the proximal *TE*/35B2 fusion fragments (20-kb *Bgl*II and 8.7-kb *Sal*I, arrows) remain unaltered. The internal fusion fragments (22-kb *Bgl*II and 16-kb *Sal*I, arrows) are lost in the single-copy *(SR100)* derivative and similar sized fragments are regained in the inverted double-copy *(SZ1)* derivative. An additional (26-kb *Bgl*II and 22-kb *Sal*I) fusion fragment is seen in the three-copy *SR36*, which is lost in the *SZ4* derivative. The *SR23* derivative has indistinguishable fusion fragments to those of the single-copy *SR100* chromosome; given that both the cytological appearance and pigment levels from the *SR23* chromosome suggest it has a greater copy number than *SR100*, it seems likely that the non-suppressible zeste phenotype may result from a triplication of the proximal copy of *TE35B* including the *TE*|35B boundary. Given the genetic fine structure of the original *TE35B(Z)* chromosome (Figure 5), the most probable structures for the derivative chromosomes are as follows: *TE35B(Z)SR100*, 35B1|w^+ rst^+|35B2; *TE35B(Z:SR100)SZ1*, 35B1|rst^+ w^+ w^+ rst^+|35B2; *TE35B(Z)SR36*, 35B1|w^+ rst^+ (w^+ rst^+) w^+ rst^+|35B2; *TE35B(Z)SR23*, 35B1|w^+ rst^+ (w^+ rst^+) w^+ rst^+|35B2 and *In(2LR)TE35B(Z:SR36)SZ4*, 35B1|w^+ rst^+ (w^+ rst^+)|43A1-35B2|w^+ rst^+|43A2. The orientation of the internal *TE* insertion, shown in parentheses, cannot be deduced from the molecular data presented for *SR36, SR23* and *SZ4*, but is consistent with their appearance in *in situ* hybridized salivary gland chromosomes (Figure 1).

red, zeste and white-sectored eyes were seen in any of the screens.

The largest class of chromosome recovered, the *GR* derivatives, gives flies with uniformly pigmented eyes. The phenotypes range from slightly dark zeste to a wild-type red eye.

Preliminary genetic characterization: Red-eyed flies can result from several different classes of mutation: (1) dominant suppressors of z^1, *i.e.* *Su*(z^1). Such mutations are common (GREEN 1967; KALISCH and RASMUSON 1974; PERSSON 1976) and were discarded. (2) Dominant suppressors of transvection between the *w* alleles carried on *TE35B(Z)*. This class included nonsuppressible *TE35B(Z)* derivatives and gross cytological aberrations with one breakpoint at, or close to, the *TE* insertion site (Table 1). (3) Mutations inactivating one of the *TE*'s two *w* genes. (4) Spontaneous, or induced, loss of half of *TE35B(Z)*. (5) Transposition of one copy of *w* to a new chromosomal site, resulting in an unpaired *w* gene (GUBB *et al.* 1986).

In the second and third series of screens, only *GR* and *GV* chromosomes carrying cytologically visible aberrations were characterized further.

Genetic crosses to distinguish the various types of mutation giving a *GR* phenotype: Unlinked *Su*(z^1) mutations were identified by their segregation from the *TE35B(Z)* chromosome. These stocks were discarded.

The remaining 69 chromosomes were expected to include both *Su*(z^1) mutations on the second chromosome and transvection-suppressing mutations mapping to the *TE*. Cytological examination of these *GR* chromosomes showed that 42 carried an aberration with one breakpoint within, or close to, *TE35B*. Twenty-seven chromosomes were superficially similar to either the progenitor *TE35B(Z)* chromosome or the smaller spontaneous half-loss forms (GUBB *et al.* 1986). Ten of these *GR* chromosomes were examined in more detail (Table 5).

To test whether the second chromosomal loci adjacent to the *TE* insertion site were affected, the 69 *GR*

TABLE 1

Cytological description of aberration-associated *TE35B(Z)GR* chromosomes

Chromosome	Cytology	FlyBase symbol
GR1	*In(2L)TE35B;*h35-h38L	*In(2L)TE35B-1*
GR3	*T(2;3)TE35B;*86A1.2	*T(2;3)TE35B-3*
GR8	*In(2L)TE35B; 35C4*	*In(2L)TE35B-8*
GR11	*In(2L)TE35B;*h35-h38L	*In(2L)TE35B-11*
GR15	*In(2LR)TE35B;*44DE	*In(2LR)TE35B-15*
GR16	*In(2L)TE35B;*h35-h38L	*In(2L)TE35B-16*
GR18	*T(Y;2)Y;TE35B*	*T(Y;2)TE35B-18*
GR25	*T(2;3)TE35B;*70B1-2	*T(2;3)TE35B-25*
GR26	*T(2;3)TE35B;*h53R-h58 + *In(3LR)*69F6-7;h53R-h58	*T(2;3)TE35B-26*
GR27	*In(2L)TE35B;*38C1.2	*In(2L)TE35B-27*
GR28	*T(2;3)TE35B;*90C3-6	*T(2;3)TE35B-28*
GR50	*T(2;4)TE35B;*101-102	*T(2;4)TE35B-50*
GR53	*In(2L)TE35B;*h35-h38L	*In(2L)TE35B-53*
GR54	*Tp(2;2)TE35B;*35C1;38F	*Tp(2;2)TE35B-54*
GR55	*In(2L)TE35B;*36C3-11	*In(2L)TE35B-55*
GR56	*In(2L)TE35B;*h35-h38L	*In(2L)TE35B-56*
GR58	*T(2;3)TE35B;*94A4-5	*T(2;3)TE35B-58*
GR200	*T(2;3)TE35B;*94A1.2	*T(2;3)TE35B-200*
GR201	*T(Y;2)Y;TE35B*	*T(Y;2)TE35B-201*
GR205	*In(2LR)TE35B;*60B8-13 + *T(2;3)*46A1-2;het + *T(Y;3)Y;*85E1.2	*In(2LR)TE35B-205*
GR206	*T(Y;2)Y;TE35B*	*T(Y;2)TE35B-206*
GR207	*T(2;3)TE35B;*h47-h53L	*T(2;3)TE35B-207*
GR208	*T(2;3)TE35B;*h35-h38L	*T(2;3)TE35B-208*
GR209	*T(2;3)TE35B;*h47-h53L	*T(2;3)TE35B-209*
GR210	*In(2L)*28B12-D1;*TE35B*	*In(2L)TE35B-210*
GR211	*T(Y;2)Y;TE35B*	*T(Y;2)TE35B-211*
GR212	*T(2;3)TE35B;*67A7-15;het;het[a]	*T(2;3)TE35B-212*
GR213	*T(Y;2)Y;TE35B*	*T(Y;2)TE35B-213*
GR214	*T(2;4)TE35B;*102C	*T(2;4)TE35B-214*
GR215	*T(2;3)TE35B;*82B	*T(2;3)TE35B-215*
GR216	*T(2;3)TE35B;*h47-h58	*T(2;3)TE35B-216*
GR217	*T(1;2)*h26-h34;*TE35B*	*T(1;2)TE35B-217*
GR218	*T(2;3)TE35B;*86E12-13	*T(2;3)TE35B-218*
GR219	*In(2)TE35B;*h35-h46	*In(2)TE35B-219*
GR220	*In(2L)TE35B;*39D	*In(2L)TE35B-220*
GR222	*In(2LR)TE35B;*41	*In(2LR)TE35B-222*
GR223	*In(2LR)TE35B;*41C	*In(2LR)TE35B-223*
GR224	*T(2;3)TE35B;*67F1.2-68A1.2	*T(2;3)TE35B-224*
GR225	*In(2L)TE35B;*h35-h38L	*In(2L)TE35B-225*
GR226	*In(2LR)TE35B;*47B10-14	*In(2LR)TE35B-226*
GR227	*In(2L)TE35B;*h35-h38L + *T(2;3)*h35-h38L;72BC + *In(3R)*h53R-h58;88B	*In(2L)TE35B-227*
GR230	*T(2;3)TE35B;*62A1[b]	*T(2;3)TE35B-230*

[a] New order: 21—*TE35B*/67A—100; 61—67A/(h35-h46) 2cen (h35-h46)/*TE35B*—60.

[b] New order: 21—21A4/26D1.2—*TE35B*/62A3—68B3.4/21A4—26C1-4/68C1.2—100;61A—62A1.2/*TE35B*—60.

chromosomes were crossed to three deletions spanning the insertion site, *Df(2L)64j, Df(2L)fn2* and *Df(2L)fn3* (Table 2). All *GR* chromosomes were viable with these deletions and expressed the *noc* phenotype associated with the *TE35B(Z)* insertion with three exceptions, *GR17, GR18* and *GR20* that carried additional lethal mutations immediately distal to *noc* (J. ROOTE, M. ASHBURNER, G. JOHNSON, D. GUBB, D. KIMBRELL and T. DAVIS, unpublished data).

***GR* chromosomes lacking gross chromosomal aberrations:** This group of chromosomes forms a heterogeneous class. Two derivatives (*GR5* and *GR19*) are similar to spontaneous single-copy *TE35B(Z)SR* derivatives being cytologically small and giving low pigment levels (Table 5). A third derivative (*GR12*) remains similar in size to the parental *TE35B(Z),* but has a low pigment level and dosage compensates as a single-copy derivative. *GR12* presumably corresponds to a double *TE* car-

TABLE 2

Description of chromosomes used for separation and mapping of aberration breakpoints

Chromosome	Cytological breakpoints
Df(2L)64j	34D1-2;35B9-C1
Df(2L)fn2	35A3;35B2
Df(2L)fn3	35B1;35B3-4
T(Y;2)A80, Dp(1;Y)B^{S} Dp(1;Y)y^{+}	35A3-4
T(Y;2)el^{4}, pr cn	35A1-2
T(Y;2)R15, Dp(1;Y)y^{+}	35B10
T(2;3)G16	35D5-7;85F6-8;87F
T(2;3)G40	35F5-7;91E5-6
T(2;4)DTD22, ast ho ed dp cl	35E1-2;101
In(2L)C163.41	27D1-2;35E1-2
In(2LR)DTD128, ho^{2}	35B1-3;48C6-8
In(2LR)noc^{4}, b noc^{4} cn bw	35B1-2;h38R-h46
In(2LR)Sco^{rv1}	35D1-2;44C4-5
Visible and lethal mutations	**Cytological locations**
b osp^{76e} Adh^{n2} rd^{s} pr cn	*osp*: 35B1.2
b el^{2} Adh^{F}	*el*: 35A4-B1
b l(2)34Fd^{CR5} Adh^{n2} pr cn	*34Fd*: 34F4-35A1
b l(2)35Aa^{4} Adh^{n2} pr cn	*35Aa*: 35A3.4
b l(2)35Bb^{1} pr	*35Bb*: 35B4
b l(2)34Fc^{6} Adh^{n4}	*34Fc*: 34F4-35A1
b wb^{SF25} Adh^{n2} pr cn	*wb*: 34F3
j^{SF7} Adh^{Uf3} rd^{s} pr cn	*j*: 34E3-5
l(2)34Fa^{1} Adh^{n1} rd^{s} pr cn	*34Fa*: 34F1.2
noc^{35Ba-2} Adh^{n11} cn vg	*noc*: 35B1.2
ms(2)34Fe^{2063}	*ms(2)34Fe*: 34F4-35A1
pu pr cn	*pu*: 35A4
rk^{4}	*rk*: 34E3-5

rying a lack of function mutation in one of its w^+ genes. The remaining derivatives (*GR2, GR9, GR17, GR20, GR21, GR23* and *GR57*) retain high pigment levels and dosage compensate as double-copy derivatives and on these criteria they correspond to *Su(z^{1})* mutations within the *TE*. Four of these (*GR2, GR9, GR21,* and *GR57*) are cytologically smaller than *TE35B(Z)* and are likely to carry small, synapsis-suppressing aberrations internal to the *TE*. The remaining three derivatives (*GR17, GR20,* and *GR23*) are cytologically similar to *TE35B(Z)*.

To test whether the *GR* chromosomes that lacked a cytologically visible breakpoint close to 35B carried dominant *Su(z^{1})* mutations, all 27 cytologically wild-type derivatives were crossed to *z^{1} w^{11E4}; TE35B(Z)/ CyO* and *z^{1} w^{11E4}; TE35BC(R)/ CyO*. In the majority of cases (22/27), progeny were phenotypically zeste. One chromosome, *GR20,* gave dark zeste eyes in *z^{1} w^{11E4}; TE35B(Z)/ TE35B(Z)GR20* flies and two, *GR2* and *GR12,* gave a posterior red crescent in a zeste eye, similar to that seen in *z^{1} w^{11E4}; TE35B(Z)/ TE35B(Z:SR100)SZ1* flies. These *GR* chromosomes, therefore, do not carry mutations in second chromosomal *Su(z^{1})* loci. They are red-eyed either because they retain only a single functional copy of w^+ or because they carry two w^+ genes together with an associated micro-aberration. The partial suppression phenotypes (*GRs 2, 12* and *20*) are similar to the partial suppression seen in aberration-associated *GR*s, presumably reflecting topological constraints on pairing between functional *w* genes.

Two exceptional derivatives, *GR9* and *GR17,* remain completely red-eyed in *TE35B(Z)/ TE35B(Z)GR* flies (Table 5) and, therefore, might carry second chromosomal *Su(z^{1})* mutations. These chromosomes were further analyzed by recombination within the second chromosome. In neither case were zeste-eyed flies recovered in over 1000 progeny, from the cross of *z^{1} w^{11E4}; GR9* or *GR17/ +* females to *z^{1} w^{11E4}; b pr sple* males. Thus neither of these *GR* chromosomes carried the zeste form of *TE35B* together with a separable *Su(z^{1})* mutation, nor a transposed single copy of *w*. In addition, *in situ* hybridization with a *w* probe showed the two sites of homology typical of the progenitor *TE35B(Z)* chromosome and confirmed the absence of additional single-copy *TE* insertions. By these criteria, *GR9* and *GR17* carry *Su(z^{1})* mutations either within *TE35B(Z)* or close to its insertion site on the second chromosome. These *Su(z^{1})* mutations might result from extreme topological constraints as in the *SR23* and *SR36* derivatives.

***GR* chromosomes associated with gross cytological breakpoints:** Forty-one cytologically aberrant derivatives of *TE35B(Z)* were recovered that partially or completely suppress the interaction between the *TE*-borne w^+ genes. All of these *GR* chromosomes have one breakpoint that is cytologically within or adjacent to the *TE* (Table 1) and one, or more, breakpoints in another chromosomal region (Table 1; Figure 4). The *GR* chromosomes include examples of most of the classes of double and multiple break events (Table 1) with the exception of deletions that remove one copy of *w* and extend either proximally or distally into the second chromosome. The lack of this class is probably not significant given that it requires one breakpoint within the *TE* and a second breakpoint close enough to the insertion site to be viable as a hypoploid (see the distribution of recovered second-site breakpoints, Figure 4).

GR aberrations were recovered with a frequency of one in 875 irradiated chromosomes. Unlike other classes of *GR*s, those associated with aberration breakpoints are predominantly partial suppressors, giving phenotypes ranging from very slightly darker than zeste to uniform red. The weakest suppressor phenotype recovered, that of *GR217,* gave a zeste eye with a posterior red crescent. Although this phenotype is uniform in *GR217* flies, it is difficult to score during screens and similar weak suppressors may well have been missed, particularly as weak suppressor phenotypes were not expected in the initial screen. If all completely red flies had been discarded in the *GR* screens, then the majority of the *GR* aberrations (36/41) would

have been retained as partial suppressors of zeste, while other classes or suppressor would have been eliminated.

As with the non-aberration-associated *GR*s, a test cross was made to the progenitor *TE35B(Z)* and the *TE35BC(R)* chromosomes. Of the 34 aberration-associated *GR* chromosomes tested, all gave a lighter eye color. In 22 cases, the eye color of z^1 w^{11E4}; *TE35B(Z)GR/TE35B(Z)* flies was completely zeste; eight cases gave dark zeste eyes (*GR*s *16, 54, 208, 212, 213, 215, 219* and *224*); two cases gave a zeste eye with red crescent (*GR*s *26* and *207*), and one case gave an orange/brown phenotype (*GR215*). The eye color of *GR* chromosomes when heterozygous with *TE35BC(R)*, in z^1 w^{11E4}; *TE35B-(Z)GR/TE35BC(R)* flies, tended to be slightly darker than when heterozygous with the original *TE35B(Z)* chromosome. In all cases, however, the addition of the single w^+ gene of *TE35BC(R)* in *trans* reduces the *zeste*-suppressing effect of a *GR* aberration breakpoint.

The two obvious models for the effect of the *GR* aberrations are either that a breakpoint falling between the two active w^+ genes prevents their transvection or, that a breakpoint inactivates one of the w^+ genes leaving the remaining single copy unsuppressed by z^1. As we will now demonstrate, both models are correct. There is, however, a third mechanism, a breakpoint to one side of both w^+ genes could cause a localized disruption in synapsis without falling between them.

Separation of aberration breakpoints: To distinguish between the above models, both elements of 12 different *GR* aberrations were separated genetically; of these, eight *GR*s corresponded to the first class. These chromosomes (*GR3, GR28, GR50, GR54, GR211, GR214, GR223* and *GR226*) carried a breakpoint within the *TE* that separated a functional copy of w^+ onto each element. The second class is represented by only one chromosome, *In(2LR)GR15,* that gives a low pigment level. This chromosome carries a single *w* gene at the 2L breakpoint. The 2R breakpoint carries rst^+ but not w^+. The *TE*-associated breakpoints of the remaining three *GR*s (*GR18, GR210* and *GR213*) were either proximal (*GR210*) or distal (*GR18* and *GR213*) to both copies of w^+.

The identity of separated *GR* elements was confirmed by mapping associated synthetic deletions in the 35B region. Where possible, this was done for both elements (*i.e., GR18, GR211* and *GR213*). In all cases, deletions had one endpoint at the *TE35B* insertion site in the *noc* locus, with the exception of *GR18,* which had a breakpoint distal to the *TE* between the *el* and *noc* loci. Thus, the synthetic deletion extending distally from the *GR18* breakpoint, in *Ts(YLt;2Lt)A80+Ts(Y;2Rt)GR18* flies, is el^- and carries the two copies of w^+ associated with *TE35B(Z).* The synthetic deletion extending proximally from the *GR18* breakpoint, *Ts(Y;2Lt)-GR18+Ts(YLt;2Rt)R15,* is el^+ noc^- and does not carry w^+ or rst^+. In this case, therefore, the transvection-disrupting breakpoint is outside *TE35B.* At the molecular level, this breakpoint falls ~30 kb distal to the *TE* insertion site (DAVIS *et al.* 1990).

In the cases of *GR3, GR15, GR28, GR50, GR54, GR211, GR214, GR223* and *GR226* the aberration breakpoint was within *TE35B* by the criterion that either w^+ or rst^+, or both, were associated with each element (Table 3).

Sites of homology to *w*: The number of GR aberrations that could be separated genetically was limited by the preexisting aberrations with similar breakpoints that were available (Table 2). An alternative approach to mapping the breakpoints with respect to the *w* genes was by *in situ* hybridization. This method is much more rapid than the genetic approach, but does not distinguish between functional and nonfunctional copies of *w.* Of the 32 *GR* aberrations examined in this way, 26 retain two sites of homology to a *w* probe (Table 4). In 17 cases the breakpoint falls between the sites of *w* homology within the *TE* (*GR*s 3, 8, 25, 26, 27, 28, 50, 54, 58, 207, 208, 211, 212, 214, 222, 223 and *226;* Table 4); in six cases (*GR*s *15, 16, 210, 218, 220* and *230*) the breakpoint falls proximal to both copies of *w* and in four cases (*GR*s *18, 213, 215* and *224*) distal to both copies of *w.* The remaining five derivatives retain only one site of *w* homology that is distal to the breakpoint in each case (*GR*s *55, 56, 219, 225* and *227*). Note that the range of suppression of the zeste phenotype is the same for the six cases in which the breakpoint falls distal to both copies of *w* (*GR*s *18, 209, 213, 215, 217* and *224*) as for the cases with medial or proximal breakpoints. There is no bias for the stronger zeste-suppressing breakpoints to fall within, or proximal, to the *TE.*

The locations of the sites of homology to *w* with respect to the chromosomal breakpoints (Figure 3 and Table 4) correlate precisely with the genetic analysis for those derivatives that have been half separated (Tables 3 and 4) with the exception of *GR15.* The *GR15* chromosome carries a single functional w^+ gene on the *Ts(2Lt)* element, but two sites of homology. A weak site of homology can also be seen on the *Ts(2Rt)* element in some chromosomes, suggesting that the breakpoint falls within the 5′ region of the proximal *w* gene (Figure 2).

More surprisingly, the chromosomal *in situ* results correlate well with the pigment levels expressed in the different *GR* derivatives. The majority of *TE35B(Z)GR* chromosomes express pigment levels similar to those of the original *TE35B(Z)* chromosome and two sites of homology to *w* (Tables 4 and 5). The *GR* chromosomes with a single site of *w* homology (*GR*s *26, 55, 56, 219, 225* and *227*) express pigment levels similar to spontaneous single-copy derivatives. Unlike the spontaneous single-copy derivatives, however, these *GR*s give only partial suppression of zeste. This suggests that the proxi-

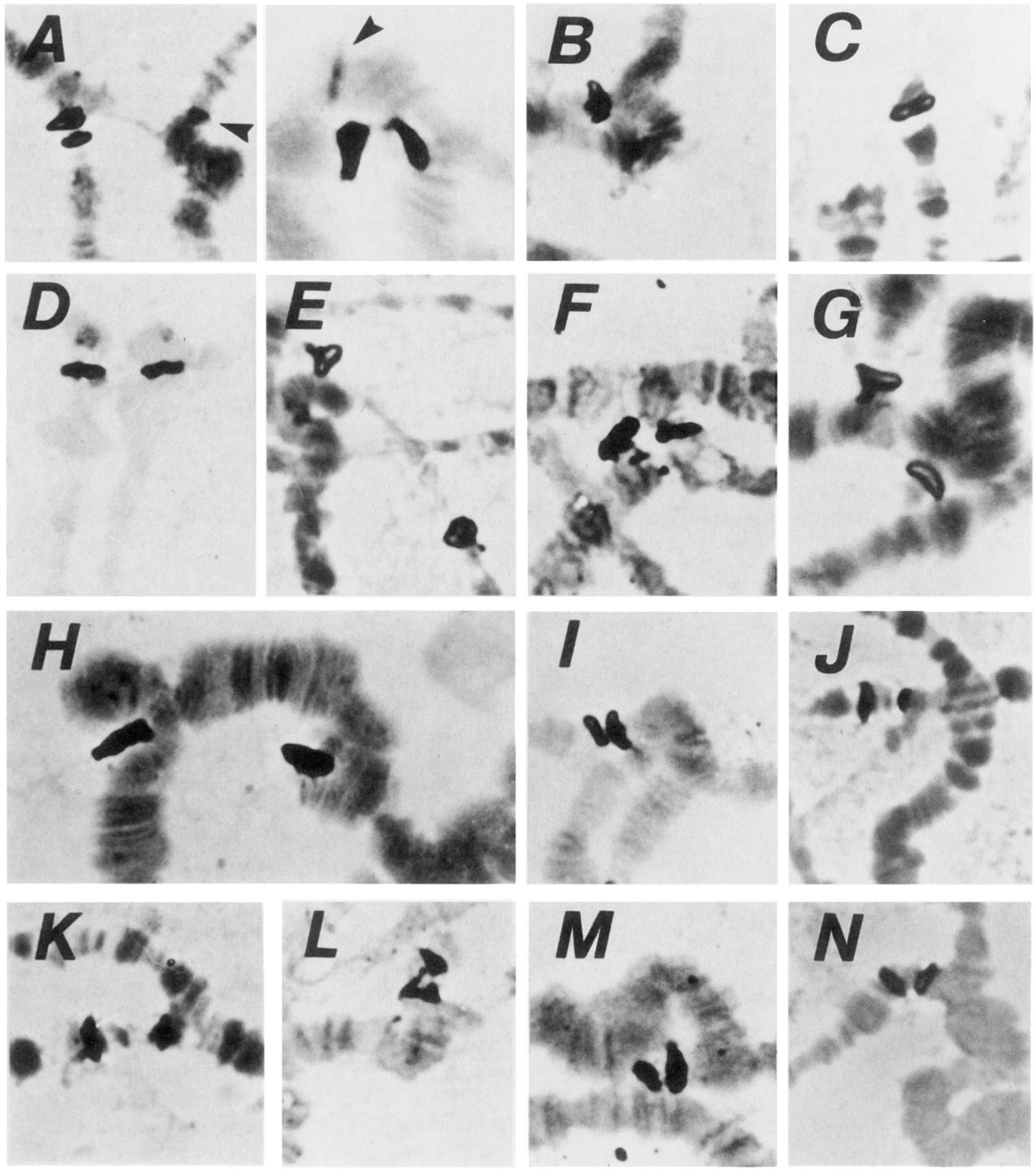

FIGURE 3.—Chromosomal *in situ*'s with a *w* probe to different *GR* chromosomes. The first row shows *GR* derivatives with only one functional *w* gene. (A) *In(2LR)GR15,* the proximal breakpoint falls close to the boundary of homology with the chromosomal probe so that two intact sites of homology are retained with a third, much weaker, site (arrowhead) visible in good preparations. Two examples are shown. (B) *In(2L)GR55* retains only the distal site of *w* homology. (C) *In(2LR)GR219* is broken within the *TE* leaving the distal site intact. The proximal site is heterochromatic and is occasionally visible in some squashes. The next five chromosomes illustrate *GR* derivatives with one breakpoint separating the two sites of homology to *w*: (D) *T(2;3)GR3,* (E) *In(2L)GR8,* (F) *T(2;3)GR28,* (G) *T(2;4)GR50,* and (H) *Tp(2;2)GR54.* Three derivatives are shown with breakpoints proximal to the *TE*: (I) *In(2L)GR210,* (J) *T(2;3)GR218,* and (K) *In(2L)GR220* and three derivatives with breakpoints distal to the *TE*: (L) *T(Y;2)GR18,* (M) *T(Y;2)GR213,* (N) *T(2;4)GR224.*

TABLE 3

Genetic analysis and pigmentation data of half-separated *GR* elements

GR	Stock	*w*	*rst*	Genetic limits	Pigment level ♂	Pigment level ♀	Ratio (♂/♀)
3	*Ts(2Lt;3Lt)GR3+Ts(2Rt;3Rt)G16*	+	−	*Df noc—CycE*	0.421	0.258	1.63
	Ts(2Lt;3Lt)G16+Ts(2Rt;3Rt)GR3	+	+	*Dp Su(H)*[a]	0.496	0.386	1.28
15	*In(2LR)GR15*^{L}*Sco*^{rv1R}	+	+	*Df noc—l(2)35Da*	0.271	0.371	0.73
	In(2LR)Sco^{rv1L}*GR15*^{R}	−	+	*Dp Su(H)*[a]	0	0	—
18	*Ts(Y;2Lt)GR18+Ts(YLt;2Rt)R15*	−	−	*Df noc—l(2)35Bg*	0	0	—
	Ts(YLt;2Lt)A80+Ts(Y;2Rt)GR18	+	+	*Df el*	ND	ND	—
28	*Ts(2Lt;3Lt)GR28+Ts(2Rt;3Rt)osp*^{90}	+	−	*Df noc—Adh*	0.441	0.312	1.41
	Ts(2Lt;3Lt)G40+Ts(2Rt;3Rt)GR28	+	+	*Dp Su(H)*[a]	0.517	0.373	1.39
50	*Ts(2Lt;4Lt)GR50+Ts(2Rt;4Rt)DTD22*	+		*Df noc—twe*	ND	ND	—
	Ts(2Lt;4Lt)DTD22+Ts(2Rt;4Rt)GR50	+	+	*Dp Su(H)*[a]	0.480	0.346	1.39
54	*Dp(2;2)GR54*	+	+	*Dp Su(H)*[a]	0.438	0.278	1.58
	Df(2L)GR54	+	−	*Df noc—lace*	0.370	0.229	1.62
210	*In(2L)GR210*^{L} *C163.41*^{R}	+	+	*Df noc—BicC*	ND	ND	—
	In(2L)C163.41^{L}*GR210*^{R}	−	ND	*Dp Su(H)*[a]	0	0	—
211	*Ts(Y;2Lt)GR211+Ts(YLt;2Rt)el*^{4}	+	ND	ND	ND	ND	—
	Ts(Y;2Lt)el^{4}*+Ts(YLt;2Rt)GR211*	+	ND	ND	ND	ND	—
213	*Ts(Y;2Lt)GR213+Ts(YLt;2Rt)el*^{4}	−	ND	ND	0	0	—
	Ts(Y;2Lt)el^{4}*+Ts(YLt;2Rt)GR213*	+	ND	ND	ND	ND	—
214	*Ts(2Lt;4Lt)GR214+Ts(2Rt;4Rt)DTD22*	+	ND	ND	ND	ND	—
	Ts(2Lt;4Lt)DTD22+Ts(2Rt;4Rt)GR214	+	ND	ND	ND	ND	—
223	*LS(2)GR223 DS(2)noc*^{4}	+	ND	ND	ND	ND	—
	LS(2)noc^{4} *DS(2)GR223*	+	ND	ND	ND	ND	—
226	*LS(2)GR226 DS(2)GR15*	+	ND	ND	ND	ND	—
	In(2LR)DTD128^{L} *GR226*^{R}	+	+	*Df elA—noc*	ND	ND	—

Pigment analysis and genetic complementation tests are incomplete for a number of elements: *In(2L)C163.41*^{L}*GR210*^{R}, *Ts(2Lt;4Lt)GR214+Ts(2Rt;4Rt)DTD22* and *Ts(2Lt;4Lt)DTD22+Ts(2Rt;4Rt)GR214* were sterile; while *Ts(YLt;2Lt)A80+Ts(Y;2Rt)GR18* carries *Bar*, which reduces the size of the eye. ND, no data.

[a] These duplications carry *Su(H)*$^{+}$ and were confirmed genetically because they enhance *Hairless* (*H*) (ASHBURNER *et al.* 1982).

mal region, at least, of both *w* genes remains intact, but that one *w* gene may be underreplicated in salivary glands as a consequence of heterochromatic position effects.

Pigment analysis: The pigment level in wild-type males and females is similar, although if allowance is made for the greater mass of female heads, the ratio of eye pigments extracted from males compared to females is 1.19:1. A single-copy w^{+} gene in a female fly (*i.e.*, w^{+}/w^{11E4}) is rather more active than in a wild-type female (w^{+}/w^{+}) giving 0.68, rather than half the amount of extractable eye pigment. This gives a ratio of 1.74:1 for extractable pigment from wild-type males (w^{+}/Y) compared to single-copy females (w^{+}/w^{11E4}). Similar ratios between the sexes are seen in single-copy *TE* strains (Table 6) although there are consistent differences between strains. Thus, a typical strain, *SR2*, gives 1.58:1 in w^{1}; *TE35B(Z)SR2/+* flies (Table 6), while *SR100* is at the high end of the range giving 2.04:1. Surprisingly, double-copy males produce greater than twice the amount of pigment as single-copy males. This hyperactivation of two ectopic copies of w^{+} in males occurs in both homozygous single-copy stocks, *e.g.*, *TE35B(Z)SR2/TE35B(Z)SR2*, and heterozygous double-copy stocks, *e.g.*, *TE35B(Z)/+* (Table 6), indicating that the effect occurs with paired *w* genes, whether in *cis* or in *trans*. This was an unexpected result, but it is consistently seen in different combinations of spontaneous *TE* derivatives (Table 6).

Hyperactivation is not suppressed by the *GR* breakpoints, even those breakpoints that separate the two sites of *w* homology (Table 4). Half-separated *GR* elements that carry a single *w* gene, however, now dosage compensate as single-copy derivatives (Table 3). Triple-copy derivatives, and three copy combinations, dosage compensate at an intermediate level between single- and double-copy derivatives (Table 6) suggesting that one copy of *w* may not hyperactivate in males. These differences indicate a complex dosage compensation response in the abnormal situation of males car-

TABLE 4

Sites of *w* homology and pigment level of aberration-associated *GR*s

Stock	Pigment level ♂	Pigment level ♀	Ratio (♂/♀)	Eye color[a]	Position of breakpoint D		Position of breakpoint P
T(2;3)GR3	1.149	0.526	2.18	3	*w*	|	*w*
In(2L)GR8	0.952	0.368	2.58	3	*w*	|	*w*
In(2LR)GR15	0.388	0.336	1.15	3	*w*	*w*	|
In(2L)GR16	0.948	0.323	2.93	4	*w*	*w*	|
T(Y;2)GR18	1.129	0.563	2.01	2	|	*w*	*w*
T(2;3)GR25	1.202	0.457	2.63	2	*w*	|	*w*
T(2;3)GR26	0.500	0.236	2.12	3	*w*	|	*w*
In(2L)GR27	1.036	0.429	2.41	3	*w*	|	*w*
T(2;3)GR28	1.057	0.372	2.84	1	*w*	|	*w*
T(2;4)GR50	1.205	0.545	2.21	2	*w*	|	*w*
Tp(2;2)GR54	1.114	0.487	2.29	5	*w*	|	*w*
In(2L)GR55	0.417	0.332	1.26	4	*w*	|	–
In(2L)GR56	0.370	0.229	1.62	3	*w*	|	–
T(2;3)GR58	1.453	0.571	2.54	3	*w*	|	*w*
T(2;3)GR207	0.813	0.330	2.46	5	*w*	|	*w*
T(2;3)GR208	0.920	0.312	2.95	5	*w*	|	*w*
T(2;3)GR209	0.770	0.340	2.26	4	|	*w*	*w*
In(2L)GR210	1.088	0.416	2.36	3	*w*	*w*	|
T(Y;2)GR211	0.981	0.652	1.50	4	*w*	|	*w*
T(2;3)GR212	1.150	0.474	2.43	5	*w*	|	*w*
T(Y;2)GR213	1.325	0.713	1.86	3	|	*w*	*w*
T(2;4)GR214	0.912	0.378	2.41	4	*w*	|	*w*
T(2;3)GR215	1.098	0.416	2.64	5	|	*w*	*w*
T(1;2)GR217	ND	ND	ND	1[b]	|	*w*	*w*
T(2;3)GR218	1.183	0.380	3.11	4	*w*	*w*	|
In(2LR)GR219	0.573	0.423	1.35	3	*w*	|	–
In(2L)GR220	0.997	0.356	2.80	1	*w*	*w*	|
In(2LR)GR222	0.950	0.394	2.41	4	*w*	|	*w*
In(2LR)GR223	0.903	0.392	2.30	3	*w*	|	*w*
T(2;3)GR224	1.010	0.415	2.43	5	|	*w*	*w*
In(2L)GR225	0.618	0.212	2.92	4	*w*	|	–
In(2LR)GR226	0.933	0.387	3.78	3	*w*	|	*w*
In(2L)GR227	0.593	0.247	2.40	4	*w*	|	–
T(2;3)GR230	1.156	0.504	2.29	2	*w*	*w*	|

Pigment data is based on a minimum of five samples of each stock. SE of these measurements were <5% in all cases. Females tend to have slightly lighter eyes than males both in the original *TE* stock and the *GR* stocks. The position of the *GR* breakpoint, |, is indicated relative to that of the proximal, P, and distal, D, sites of *w* homology within the *TE*-, no site of homology.

[a] Eye color of $z\ w^{11E4}$; *GR*/+ genotypes assessed on a range from 5 to 0 (5 = red; 4 = purple; 3 = orange/brown; 2 = orange; 1 = dark zeste; 1* = zeste with a red crescent at posterior edge of eye; 0 = zeste).

[b] The pigment level of *GR217* could not be measured in a z^1 background as the *X* chromosome in *T(1;2)GR217* carries z^1. In a $z^1\ w^{11E4}$ background, *GR217* gives a zeste eye with a red crescent.

rying more than a single *w* gene. Whatever the molecular basis, these results are internally consistent and the aberrant response is insensitive to disruption by the *GR* breakpoints in a z^+ background.

TABLE 5

Cytological appearance and pigment level of non-aberration-associated *GR*s

Stock	Pigment level Male	Pigment level Female	Ratio	Eye color[a]	Cytological description
GR2	1.216	0.512	2.38	5	Like *TE35B(Z)*
GR5	0.401	0.250	1.60	5	Small *TE*
GR9	0.924	0.414	2.23	5	Compact 3 band *TE*
GR12	0.550	0.380	1.45	3	Like *TE35B(Z)*
GR19	0.315	0.232	1.36	5	Small *TE*
GR17	0.734	0.386	1.91	5	Like *TE35B(Z)*
GR20	1.364	0.490	2.78	5	Like *TE35B(Z)*
GR21	1.294	0.514	2.52	3	Small *TE*
GR23	0.879	0.425	2.07	3	Like *TE35B(Z)*
GR57	1.065	0.427	2.50	5	Small *TE*

[a] Eye color of $z^1\ w^{11E4}$; *GR*/+ genotypes assessed on the same scale as in Table 4. Pigment data is based on a minimum of five samples of each stock in a $z^+\ w^{11E4}$ background. SE in these measurements were <5% in all cases.

Molecular mapping of the GR breakpoints: Southern blots of DNA from 32 aberration-associated *GR* chromosomes digested with *Sal*I or *Bgl*II identified six with breakpoints within the 5.4-kb *Bgl*II *Sal*I fragment that includes the first three exons of the *w* gene and extends 1.3 kb in the 3′ direction (*GR15, GR56, GR211, GR212, GR215* and *GR224*). In addition *GR219* and *GR217* are broken within the 10.7- and 11-kb *Bgl*II *Sal*I fragments corresponding, respectively, to the distal and internal genomic *TE* fragments including the 5′ end of the *w* gene (data not presented).

Distribution of the second-site breakpoints of the GR aberrations: Each *GR* has one breakpoint at 35B1.2 within, or adjacent to, *TE35B(Z)*. In most cases, the second breakpoint of *GR* inversions is either proximal to the *TE* on chromosome arm 2L or on the 2R. Five inversion breakpoints fall between 35C and 39D (*In(2L)s GR8, GR27, GR54, GR55* and *GR220*). Four breakpoints fall between 41C and 60B8-13 in 2R euchromatin (*In(2LR)s GR15, GR205, GR223* and *GR226*). In addition there are 10 breakpoints in the centric heterochromatin, six in 2L heterochromatin (*In(2L)s GR11, GR16, GR53, GR56, GR225* and *GR227*) and three in 2R heterochromatin (*In(2LR)s GR219, GR222* and *GR225*). Only one *GR* was recovered with a second breakpoint distal to *TE35B, In(2L)GR210*. The low frequency of breakpoints distal to the *TE* must result from a selective bias in recovery. Inversions with one breakpoint between 34F and 35D, and the other more distally on the 2L are readily recovered in screens not based on transvection. Of 19 such inversions, 12 have the second breakpoint distal to 35B and seven have proximal euchromatic breakpoints (ASHBURNER *et al.* 1983 and unpublished observations). LINDSLEY *et al.* (1972) found

TABLE 6

Pigment level of spontaneous *TE* derivatives

Stock	Pigment level		Ratio	Copies of w^+	Eye color[a]
	Male	Female			
TE35B(Z)SR2/+	0.405	0.258	1.58	1	5
TE35BC(R)/+	0.410	0.242	1.69	1	5
TE35B(Z)SR100/+	0.295	0.145	2.04	1	5
TE35B(Z)/+	1.067	0.470	2.27	2	0
TE35B(Z;SR100)SZ1/+	0.923	0.428	2.16	2	0
TE35B(Z)SR100/TE35B(Z)SR100	0.894	0.402	2.22	2	0
TE35B(Z)/TE35B(Z)SR100	1.212	0.636	1.91	3	0
TE35B(Z)/TE35BC(R)	1.196	0.838	1.43	3	0
TE35B(Z)SR23/+	1.079	0.752	1.43	3	5
TE35B(Z)SR36/+	1.267	0.708	1.79	3	5
TE35B(Z:SR36)SZ4/+	1.248	0.692	1.80	3	0

Pigment data is based on a minimum of five samples of each stock. SE of these measurements were <5% in all cases.

[a] Eye colors in a z^1 w^{11E4}; *GR/+* genetic background were either red (5) or zeste (0) in both sexes.

that the majority of X-ray-induced *T(Y;2)* translocation breakpoints in 2L euchromatin (57/72) fall distal to 35B (Figure 4).

DISCUSSION

The *w-w* interaction and transvection: The interaction of *w* genes in z^1 w^+/z^1 w^+ flies is an example of transvection as defined by LEWIS (LEWIS 1954; JACK and JUDD 1979). The *w-w* interaction occurring within *TE35B(Z)* or the tandem duplication z^1 *Dp(1;1)*w^{+R}, investigated by JACK and JUDD (1979), is formally a *cis* interaction in the sense that both copies of *w* are carried in tandem on the same homologue. The phenomenology of the interaction is, however, distinct from that defined by LEWIS (1954) as cisvection, or position effect variegation (HENIKOFF 1990). Not only do combinations of single- and double-copy derivatives fail to distinguish between *cis* and *trans* regulatory effects, but aberration breakpoints either proximal or distal to both copies of *w* can suppress the *w-w* interaction. These observations imply that the interaction is proximity dependent rather than requiring physical continuity of the chromosome. In this sense the phenomenon reflects a *trans*-regulatory rather than a *cis*-regulatory interaction. This conclusion is confirmed by the finding that the suppression phenotype of most *GR* aberrations is only partial (Table 4). Of the 17 GR aberrations with breakpoints between the *w* genes, 15 give only partial suppression of the *w-w* interaction, despite the impossibility of *cis* interactions occurring across the chromosomal breakpoints (Table 4).

A number of lines of evidence suggest that the *w-w* interaction occurs over an extremely short range. First, suppression does not occur with the triple-copy derivatives *TE35B(Z)SR36* and *SR23*, although synthetic three-copy combinations give a zeste phenotype. The probable explanation is that the triple-copy *TE* derivatives are too compact to allow the chromosomes to form a double loop bringing the zeste-binding domains of all three *w* genes into contact. The topological constraint might, however, be released by an aberration breakpoint. This seems to be the explanation for the zeste phenotype of the *SZ4* derivative. The *In(2LR)TE35B-4, TE35B-(Z:SR36)SZ4* chromosome was recovered as a spontaneous inversion within *TE35B(Z)SR36* that carries the most proximal copy of *w* to 43A1-2 (Figure 1G) allowing all three copies of *w* to synapse.

This interpretation implies that the *w-w* interaction occurs over a much shorter range than the linear distance between the two copies of *w* on the *TE35B(Z)* chromosome. Before discussing our results in more detail, we will review what is known of the fine structure of different forms of *TE35B*.

The genetic fine structure of spontaneous *TE35B* derivatives and their zeste phenotype in various combinations: Previous work has indicated that the original *TE35B* chromosome carried a tandem duplicated form of the *TE* with the genetic order 35B1 | w^+ rst^+ w^+ rst^+ | 35*B2* (GUBB *et al.* 1986; LOVERING *et al.* 1991). This structure is confirmed by the genetic mapping of the half-separated elements of the *TE35B(Z)GR* aberrations (Table 3 and Figure 5). In adult flies, the heterozygous *TE35B(Z)* chromosome gives a uniform *zeste* eye color, in a z^1 w^{11E4} background, indistinguishable from the zeste phenotype of z^1 w^+ females. By this criterion, the duplicated *w* genes are tightly synapsed, presumably in an inverted loop structure. Such an inverted loop would be expected to cause a localized topological stress at the insertion site, but if this does occur in the pigment cells of the eye, it is insufficient to affect the *w-w* interaction. In salivary gland polytene chromosomes the

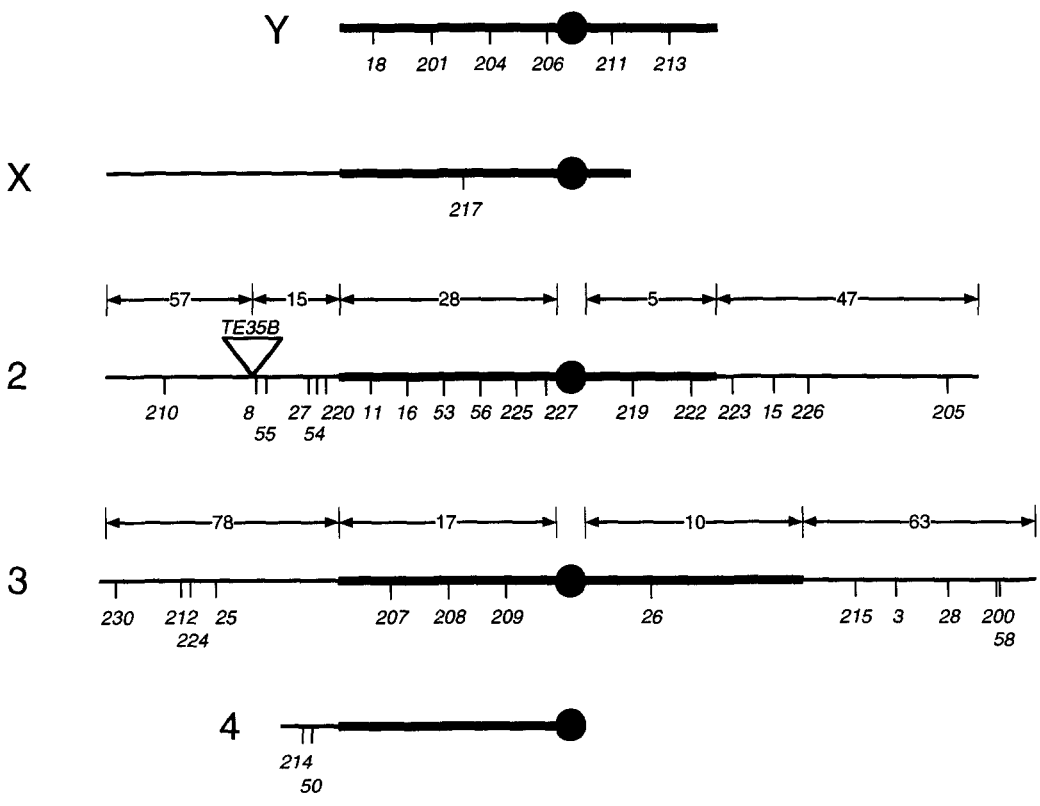

FIGURE 4.—Distribution of the second-site breakpoints of the *GR* aberrations. The second site breakpoints are uniformly distributed in euchromatic regions of other chromosome arms with the exception of the 2L arm, which has only one second-site breakpoint distal to the *TE*. The proximal region of the 2L has been drawn on an expanded scale and the distal region on a contracted scale to accommodate all the second-site breakpoints. The numbers on the horizontal bars give the numbers of breakpoints recovered within the equivalent regions of the second chromosome by LINDSLEY and SANDLER *et al.* (1972). The order of *GR* breakpoints within heterochromatic regions (solid bars) is unknown except that 2L and 2R heterochromatic breakpoints were distinguished by genetic tests.

w sequences are forced into a V structure by pairing to the wild-type homologue (Figure 1B). Homozygous *TE35B(Z)/TE35B(Z)* chromosomes would pair homologously across the whole region without distortion at the *TE* boundaries.

The increased stability resulting from not having to form an inverted loop might also stabilize the synapsis of *GR* derivatives when paired with the original *TE35B(Z)* chromosome. In fact only *GR9* and *GR17* retain their original eye-color in z^1 w^{11E4}; *TE35B(Z)GR/TE35B(Z)* flies. The other *GR*s give much weaker phenotypes in this combination: 44 are completely zeste, four give a zeste eye with a posterior red crescent and nine a uniform dark zeste eye.

The inverted repeat form of the *TE (SZ1)* has the fine-structure 35B1 | rst^+ w^+ w^+ rst^+ | 35B2 (GUBB *et al.* 1986) and gives a hairpin loop structure in heterozygous salivary gland chromosomes and a uniform zeste eye color in z^1 w^{11E4}; *TE35B(Z:SR100)SZ1/+* flies (Figure 1C). Both the original and inverted forms of the *TE* synapse closely with single-copy derivatives to give a zeste phenotype, in z^1 w^{11E4}; *TE35B(Z)/TE35B(Z)SR100* and z^1 w^{11E4}; *TE35B(Z:SR100)SZ1/TE35B(Z)SR100* flies (Table 6 and GUBB *et al.* 1986). In heterozygotes between the tandem and inverted forms of the *TE*, however, the *w* genes would form a tight cluster containing three copies in one orientation, with the fourth in reversed orientation (Figure 1D). The four *w* genes could only stack in register if the chromosome were sufficiently flexible to allow an inverted loop within the extent of a single-copy *TE* (estimated at 350 kb for *TE35B(Z)SR100* by LOVERING 1988). This distance appears to be close to a critical limit as the phenotype of z^1 w^{11E4}; *TE35B(Z)/TE35B(Z:SR100)SZ1* flies is zeste with a crescent of red cells in the posterior region of the eye. This phenotype is not simply a consequence of having four adjacent *w* genes as other four copy combinations, such as *TE35B(Z)/TE35B(Z)* and *In(2LR)-TE35B(Z:SR36)SZ4/TE35B(Z:SR100)*, give a uniform zeste eye. Instead the red crescent phenotype corresponds to the weakest Su(z^1) phenotype that can be distinguished, as seen in double-copy *TE35B(Z)GR217/+* flies and in five of the *GR* strains that do not become completely zeste eye in four copy combination with *TE35B(Z)* (in z^1w^{11E4}; *TE35B(Z)/TE35B(Z)GR* flies).

The interpretation of the *zeste* phenotypes of the spontaneous *TE35B* derivatives presented above assumes that the sites of homology to the *w* probe indicate active *w* genes. Two lines of evidence support this. First, the pigment levels of the different derivatives (Table 4) correlate with the number of sites of homology to the probe. Second, when probed with a 10-kb genomic *w* clone carrying the 5′ region necessary for the z^1-mediated interaction to occur (HAZELRIGG *et al.* 1984), most

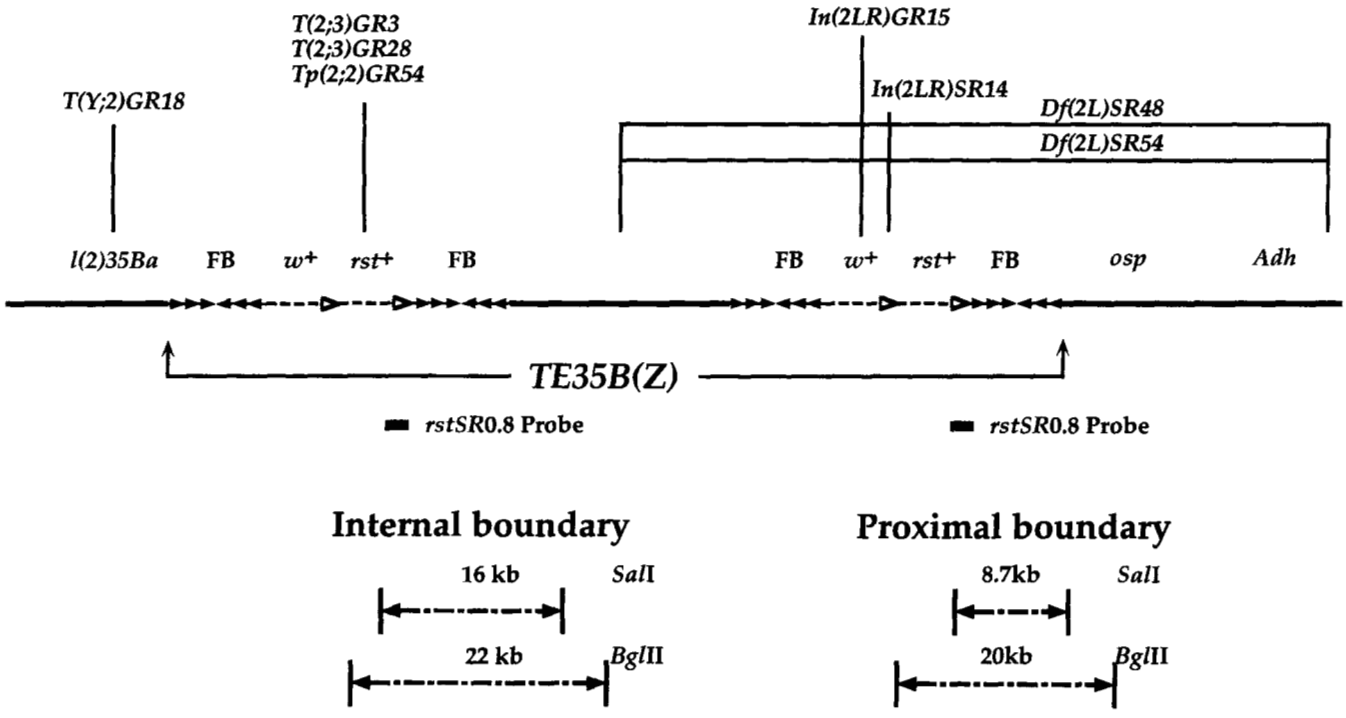

FIGURE 5.—Genetic fine structure of *TE35B(Z)*. The *TE* consists of a tandem duplication of ISING's original element that carried w^+ and rst^+ flanked by *FB* elements. The order of the *X* chromosome loci can be deduced from the breakpoints of spontaneous and gamma ray-induced red-eyed derivatives of *TE35B(Z)*. The presence of two copies of rst^+ within the progenitor *TE35B(Z)* could not be assayed by either genetic or biochemical tests. However, both elements of *In(2LR)GR15* carry rst^+ as do all spontaneous half-loss *TE35B(Z)SR* derivatives (GUBB *et al.* 1986). The breakpoint of *GR15* is within the proximal *w* gene leaving w^+ and rst^+ at the *In(2LR)GR15*^{L} breakpoint. The most distal locus within the *TE* is w^+ as both *T(2;3)GR3* and *T(2;3)GR28* carry w^+, but not rst^+ on their *Ts(2Lt)* element, and w^+ and at least one copy of rst^+ on their *Ts(2Rt)* element. Similarly, *Tp(2;2)GR54* retains w^+, but not rst^+, on the deletion element and at least one copy of rst^+ on the duplication element. The most proximal locus within the *TE* is rst^+ as the spontaneous derivative *In(2LR)TE35B-14* carries rst^+, but not w^+, at its right breakpoint while retaining rst^+ and two copies of w^+ at its left breakpoint (GUBB *et al.* 1986).

of the derivative chromosomes showed no restriction fragment polymorphisms (data not presented). There were eight exceptions carrying breakpoints within, or immediately adjacent, to the *w* locus (*GRs 15, 58, 211, 212, 215, 217, 219* and *224*).

Chromosomal synapsis and partial suppression of *w-w* interactions: Our working model is that the disruption of synapsis in the immediate vicinity of a breakpoint can spread a short distance within the *TE*. Such distortions might stress the chromatin structure and separate the 5′ regions of the *w* transcription units. In most cases, the suppression of the *w-w* interactions would be partial, explaining the range of modified zeste phenotypes described in this study. An alternative view is that the modification of chromatin structure in the vicinity of a breakpoint interferes with the binding of the neomorphic ZESTE-1 protein and relaxes the inhibition of transcription of *w* (PIRROTTA 1991). The two views are not necessarily exclusive as the repression of *w* transcription by the z^1 product is dependent on the proximity of two or more *w* genes. In either case, the localized disruption of homologous pairing by a breakpoint in the vicinity of the *TE* would provide a torsional stress that would inhibit pairing between *w* genes over a short range. In this context, it is interesting that oligomers of the ZESTE protein can bind two or more DNA sites simultaneously *in vitro* (PIRROTTA 1991), which suggests that the protein might form short-range bridges between adjacent zeste-binding sites in native chromatin. The role of zeste might be to facilitate chromosomal looping bringing together enhancer-bound factors and the transcription complex (PIRROTTA 1991). The ZESTE-1 protein might stabilize an abnormal looping configuration between the enhancer and promoter regions of adjacent copies of *w* that had the effect of suppressing transcription. Such configurations would be particularly sensitive to the torsional stress associated with a localized disruption of synapsis. A significant result for this class of model is that null alleles of *z* act as recessive enhancers of position effect variegation suggesting that the ZESTE protein promotes changes in chromatin configuration *in vivo* (JUDD 1995).

Whatever the mechanism, the range over which an aberration breakpoint can suppress the *w-w* interaction is of the order of a few hundred kilobases, or less, in

most cases falling within the *TE* itself. In 17 of the 28 *GR* chromosomes that retain two sites of *w* homology and carry cytologically visible aberrations, the breakpoint falls between these sites (Table 4). This separation, equivalent in size to a single-copy *TE*, has been estimated as being ~350 kb for *TE35B(Z)SR100* (LOVERING 1988). Six *GR*s break proximal to both copies of *w* and five distal to both copies. None of the *GR* chromosomes are associated with mutations in the adjacent autosomal loci, with the exception of *GR17* and *GR20*, which give completely red eyes (Table 5). In addition, one derivative *GR18* was shown to have a breakpoint between the *el* and *noc* loci, 30 kb distal to the *TE* insertion site (DAVIS *et al.* 1990), and eight have breakpoints either within *w* or within 12 kb of its genomic boundaries. Thus, the great majority of *GR* breakpoints fall within the confines of the *TE* itself. These results are comparable to the study of SMOLIK-UTLAUT and GELBART (1987) in which they recovered four breakpoints proximal and a single breakpoint distal to *w* at its normal chromosomal location of 3C2 that suppress the zeste phenotype.

Perhaps the most surprising feature of our results was the recovery of 15 *GR* breakpoints falling between the sites of *w* homology, but giving only partial suppression. An explanation of this result is suggested by the close synapsis seen in salivary gland chromosomes. In cytological squashes, aberrant chromosomes may pair along their entire length with their wild-type homologues. As a consequence, *GR* breakpoints would tend to be held together by the intact wild-type homologue leading to partial synapsis of the *w* genes.

Even when the *GR* breakpoint falls within one of the *w* genes the suppression of the *w-w* interaction may be incomplete. Thus, the *GR15, GR211, GR219* and *GR217* breakpoints interrupt one of the *w* genes, but none of these stocks express completely red eyes (Table 4 and legend). These *GR*s are analogous to the z^1 *Dp(1;1)*w^{+R} chromosome in showing suppression of a single complete *w* gene (GREEN 1967) although, in the case of the *GR* chromosomes, the suppression is partial.

Although we have been able to demonstrate only one cytologically visible *GR* breakpoint (*GR18*) within second chromosome sequences adjacent to the insertion site, among the 45,285 flies screened, our model would predict that the localized disruption in pairing would be transmitted across the *TE* boundaries over a similar range to that within the *TE* itself. This being the case, breakpoints within the 35B1.2 region of the homologous second chromosome should cause a similar disruption of the *w-w* interaction. This class of transvection-suppressing breakpoints has been described by GUBB *et al.* (1990) in z^1 w^{11E4}; *GT/TE35B(Z)* flies, where *GT* corresponds to a chromosomal rearrangement with one breakpoint between 35A1 and 35B2. The frequency of recovery of *GT* breakpoints is low (about one in 10,000 chromosomes, GUBB *et al.* 1990), but the phenomenon was well characterized using preexisting aberrations. Of 87 chromosome rearrangements with breakpoints in the 35A1 to 35C2 interval, 40 act as partial suppressors of the *w-w* interaction when heterozygous with *TE35B(Z)*. None of these 40 breakpoints, nor the nine novel *GT* aberrations, affect the inverted hairpin loop form of the *TE*, which remains zeste eyed in z^1 w^{11E4}; *GT/TE35B(Z:SR100)SZ1* flies. By this criterion, the inverted repeat (35B1 | rst^+ w^+ w^+ rst^+ | 35B2) form of the *TE* is more stably synapsed than the tandem repeat (35B1 | w^+ rst^+ w^+ rst^+ | 35B2) form in the pigmented cells of the eye. This difference in stability between the two duplicated forms of the *TE* is strong confirmation that the *w-w* interaction is affected by extremely localized topological constraints.

The relationship between gene expression and levels of extractable eye pigment: In this study, pigment assays have been used to give an objective assessment of the eye color phenotype. It was not expected that these measurements would give a reliable indication of the transcriptional activity or number of *w* genes in the *TE35B(Z)* derivatives. Our results, however, demonstrate a clear correlation between pigment levels and the number of functional *w* genes (Tables 3–5). This is an empirical correlation, but it is consistent with the number of sites having homology to a *w* probe for most of the *TE35B(Z)GR* derivatives (Table 4) and to the number of active *w* genes in *TE* combinations (Table 6) and half-separated elements (Table 3). Part of the reason for this regularity is that the *TE* is insensitive to chromosomal position effects at its insertion site (ISING and BLOCK 1981) presumably because of its large size. Whatever the reason, ISING's large collection of *TE* insertion strains do not show the wide range of phenotypes typical of *P*{w^+} mini-gene constructs (HAZELRIGG *et al.* 1984).

Dosage compensation, synapsis and the *zeste* reaction: The dosage compensation mechanism ensures that *X*-linked genes are expressed at similar levels in males and females. In Drosophila, both *X* chromosomes are transcriptionally active in females, but at half the level in males (see LUCCHESI 1973 for review). In the abnormal situation where a female carries a single copy of an *X*-linked gene heterozygous with a deletion, the single copy produces rather more than half the normal level of gene product. Conversely, males carrying a duplication express less than double the normal level of gene product (SEECOF *et al.* 1968). Our results show that the ratio of eye pigments extracted from males:females is within the expected range for single-copy *TE35B* derivatives (*e.g.*, 1.58:1 for *TE35B(Z)SR2*). A much higher ratio, however, is seen with the double-copy derivatives (2.27:1 for *TE35B(Z)*).

The high male:female ratio is retained in most *GR* stocks but lost in those that have low pigment levels

(Tables 4 and 5). The different ratios do not reflect simply a differential response of males and females to increased levels of *w* product, but are dependent on the number of copies of the *w* gene. Thus, single copies of *w* from the separated elements of the *GR3, GR28, GR50* and *GR54* chromosomes (Table 3) give male: female pigment ratios similar to the single-copy derivative *TE35B(Z)SR2*, or the wild-type gene (*i.e.*, w^+/Y: w^+/w^{11E4}). The original stocks of *GR3, GR28, GR50* and *GR54*, however, each retaining two functional copies of *w*, continue to give male:female ratios similar to that of the parental *TE35B(Z)* chromosome (Tables 4 and 6). Conversely, homozygous single-copy, *TE35B(Z)SR100/ TE35B(Z)SR100* flies express a higher male:female ratio than heterozygous, *TE35B(Z)SR100/+* flies (Table 6). These results were completely unexpected, but presumably involve the dosage compensatory regulatory element immediately 5′ to the *w* gene (HAZELRIGG *et al.* 1984; PIRROTTA *et al.* 1985). The pigmentation data are internally consistent and indicate that the dosage compensation mechanism is sensitive to the numbers of copies of *w*. Some type of *w-w* interaction is occurring even in a z^+ background.

The corollary to this conclusion is that the z^1-mediated suppression of *w* might result from abnormal regulation of the mechanism of dosage compensation. The zeste phenotype itself is unaffected by increasing numbers of *w* genes or by sex-limited dosage compensation effects. Thus, up to five copies of *w*, in z^1 w^{11E4}/Y; *In(2LR)TE35B-4, TE35B(Z:SR36)SZ4/ TE35B(Z)* males, give a zeste phenotype that is indistinguishable from that given by z^1 w^{11E4}; *TE35B(Z)/+* females or, indeed z^1 w^+ females, with two wild-type *w* genes that are more active than the w^a-revertant alleles carried by the *TE*. This is particularly surprising as the *GR* breakpoints show that a wide range of zeste phenotypes are possible.

Chromosome replication and synapsis at *bx, dpp* and *eya:* There are two major differences between the suppression of the *w-w* interaction in a z^1 background and transvection-suppression at the other loci that have been well characterized, *bithorax* (*bx*) and *decapentaplegic* (*dpp*) and, to a lesser extent, *eyes absent* (*eya*). First, at these three loci, transvection-suppressing breakpoints prevent partial complementation between mutant alleles, while at *w* the response is downregulation of the activity of wild-type alleles. Second, the "critical regions" within which transvection-suppressing breakpoints fall (LEWIS 1954) extend proximally from the transvection-sensitive locus over a large region of the chromosome arm, between 89E and the centromere for *bx,* 22F and 35DE for *dpp* and 26EF, and, at least, 33BE for *eya* (LEWIS 1954; GELBART 1982; LEISERSON *et al.* 1994). Such long range effects on synapsis would not be expected from localized pairing constraints at the aberration breakpoints. The partial complementation between some structurally modified alleles of all three of these genes is, however, affected by z^a mutations (KAUFMAN *et al.* 1973; GELBART and WU 1982; LEISERSON *et al.* 1994), a feature that they share with some partially complementing alleles of *w* (BABU and BHAT 1980; JUDD 1995). In addition, the distribution of second-site breakpoints of transvection-suppressing aberrations is nonrandom for *bx* and *dpp* (LEWIS 1954, GELBART 1982) and, as we show in this paper, for *TE35B.* These similarities imply that the phenomenon of transvection at these loci must be related to that at *w.*

To explain the size of the *bx* critical region and the observation that transvection-suppressing breakpoints always fall proximal to the *bx* locus, LEWIS (1954) postulated that somatic pairing is initiated at the centromere and spreads distally. Breakpoints within the critical region could disturb the progression of homologous pairing along the chromosome arm and affect synapsis of over an extended region. GELBART (1971, 1982) suggested a similar model in which the sites of initiation of chromosomal pairing corresponded to the chromosomal constrictions visible in salivary gland chromosomes. The critical region for *w* would be bounded by the 3C4 constriction, that of *dpp* by the 35DE constriction and that of *bx* by the centromere. In both these variants of a dynamic pairing model, in which synapsis is spreading along a chromosome, aberrations would reduce the proportion of the cell cycle during which transvection-sensitive loci were closely synapsed. This effect is likely to be most significant during the rapid embryonic division cycles, but should not modify the expression of the *w* gene during the pupal stage, even for the *TE89E* that is inserted immediately proximal to *bx* (SMOLIK-UTLAUT and GELBART 1987). The idea that the degree of pairing disruption might be influenced by cell division rate has been proposed by GOLIC and GOLIC (1996).

The hypothesis that the restricted critical region for *w* is a consequence of its normal location close to the 3C4 constriction was tested by SMOLIK-UTLAUT and GELBART (1987) who found that a deletion that removed the 3C4 constriction did not extend the proximal boundary of the *w* critical region. In addition, the *w-w* interaction between *TE*s transposed to the *bx* and *dpp* regions remains unaffected by aberrations recovered as suppressors of transvection of *bx* and *dpp* (SMOLIK-UTLAUT and GELBART 1987). Thus, the restricted critical region of *w* is a feature of the gene itself and not dependent on its chromosomal location. These results are confirmed by our results with *TE35B(Z).* In particular, the critical region for *w* genes in 35B1-2 does not extend proximally to the 35DE boundary of the *dpp* critical region. Furthermore, we show that transvection-disrupting breakpoints can fall either proximal or distal to the ectopic insertion site.

By these criteria, the short-range disruption of synap-

sis of *w* genes appears to be qualitatively distinct from the long-range polar effects of breakpoints proximal to *bx, dpp* and *eya* (LEWIS 1954; GELBART 1982; GELBART and WU 1982; SMOLIK-UTLAUT and GELBART 1987; LEISERSON *et al.* 1994). As a corollary to this, although chromosome deletion end-points close to the insertion of *TE35B* can cause localized disruption of synapsis of *w* (GUBB *et al.* 1990), deletions are not recovered in screens for suppressors of transvection at *bx, dpp,* or *eya* (LEWIS 1954; GELBART 1982; GELBART and WU 1982; SMOLIK-UTLAUT and GELBART 1987; LEISERSON *et al.* 1994). Deletions that were large enough to disrupt long-range polar effects would be grossly hypoploid and cause aneuploid lethality.

It may well be that transvection between synapsed genes is a more general phenomenon in Drosophila than has been recognized. The adult phenotype of most genes will depend on expression during the pupal and adult stages, when cellular division rates are low. Only genes involved in fate-determination during the rapid divisions in early development might be affected by long-range polar effects.

A related synapsis-dependent phenomenon has been described for dominant heterochromatic inactivation of *brown* (HENIKOFF 1994). The inactivation of bw^+ by bw^D depends on an ectopic block of centric heterochromatin on the bw^D chromosome being able to pair back to the centromere (HENIKOFF *et al.* 1995; CSINK and HENIKOFF, 1996; DERNBERG *et al.* 1996). Although this process demonstrates that nuclear architecture can be perturbed over long distances by chromosomal breakpoints, the effect would be static in cells that are not dividing rapidly, unlike the LEWIS (1954) and GELBART (1982) models. As with the short-range effect at *w*, the disruption of dominant heterochromatic inactivation at *bw* is not polar, breakpoints can be either proximal or distal to *bw* on either homologous chromosome.

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LITERATURE CITED

ASHBURNER, M., 1982 The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for Alcohol Dehydrogenase III. Hypomorphic and hypermorphic mutations affecting the expression of Hairless. Genetics **101:** 447–459.

ASHBURNER, M., C. DETWILER, S. TSUBOTA and R. C. WOODRUFF, 1983 The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase. VI. Induced revertants of Scutoid. Genetics **104:** 405–431.

BABU, P., and S. G. BHAT, 1980 Effect of zeste on white complementation, pp. 35–40 in *Development and Neurobiology of Drosophila,* edited by O. SIDDIQI, P. BABU, L. M. HALL and J. C. HALL. Plenum Press, New York.

BINGHAM, P. M., and B. H. JUDD, 1981 A copy of the *copia* transposable element is very tightly linked to the w^a allele at the *white* locus of *Drosophila melanogaster.* Cell **25:** 705–711.

CHIA, W., S. MCGILL, R. KARP, D. GUBB and M. ASHBURNER, 1985 Spontaneous excision of a large transposable element of *Drosophila melanogaster.* Nature **316:** 81–83.

CLANCY, C. W., 1942 The development of eye colors in *Drosophila melanogaster.* Further studies on the mutant claret. Genetics **27:** 417–440.

CRAYMER, L., 1981 Techniques for manipulating chromosomal rearrangements and their application to *Drosophila melanogaster.* 1. Pericentric inversions. Genetics **99:** 75–97.

CSINK, A. K., and S. HENIKOFF, 1996 Genetic modification of heterochromatic association and nuclear organization in *Drosophila.* Nature **381:** 529–531.

DAVIS, T., J. TRENEAR and M. ASHBURNER, 1990 The molecular analysis of the *el-noc* complex of *Drosophila melanogaster.* Genetics **126:** 105–119.

DERNBURG, A. F., K. W. BROMAN, J. C. FUNG, W. F. MARSHALL, J. PHILIPS *et al.,* 1996 Perturbation of nuclear architecture by long-distance chromosome interactions. Cell **85:** 745–759.

GANS, M., 1953 Étude génétique et physiologique du mutation z^1 de *Drosophila melanogaster.* Bull. Biol. Fr. Belg. **38** (Suppl): 1–90.

GANS-DAVID, M., 1949 "*zeste*" *(z):* mutation lié au sexe et limité par le sexe chez *Drosophila melanogaster.* 1. Étude génétique de z méchanisme de la limitation par le sexe. Bull. Biol. Fr. Belg. **83:** 136–157.

GEHRING, W. and R. PARO, 1980 Isolation of a hybrid plasmid with homologous sequences to a transposing element of *Drosophila melanogaster.* Cell **19:** 897–904.

GELBART, W. M., 1971 Cytogenetics of zeste expression in *Drosophila melanogaster.* Ph.D. Thesis, University of Wisconsin, Madison.

GELBART, W. M., 1982 Synapsis-dependent allelic complementation at the decapentaplegic complex in *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA **79:** 2636–2640.

GELBART, W. M., and C-T. WU, 1982 Interactions of zeste mutations with loci exhibiting transvection effects in *Drosophila melanogaster.* Genetics **102:** 179–189.

GOLIC, M. M. and GOLIC, K. G., 1990 A quantitative measure of the mitotic pairing of alleles in Drosophila melanogaster and the influence of structural heterozygosity. Genetics **143:** 385–400.

GREEN, M. M., 1959 Spatial and functional properties of pseudoalleles at the white locus in *Drosophila melanogaster.* Heredity **13:** 303–315.

GREEN, M. M., 1967 Variegation of the eye color mutant zeste as a function of rearrangements at the white locus in *Drosophila melanogaster.* Biol. Zentralbl. **86** (Suppl.): 211–220.

GUBB, D., M. SHELTON, J. ROOTE, S. MCGILL and M. ASHBURNER, 1984 The genetic analysis of a large transposing element of *Drosophila melanogaster.* The insertion of a w^+ rst^+ TE into the *ck* locus. Chromosoma **91:** 54–64.

GUBB, D., J. ROOTE, G. HARRINGTON, B. DURRANT, S. MCGILL *et al.,* 1985 A preliminary genetic analysis of *TE146,* a very large transposing element of *Drosophila melanogaster.* Chromosoma **92:** 116–123.

GUBB, D., J. ROOTE, S. MCGILL, M. SHELTON and M. ASHBURNER, 1986 Interactions between *white* genes carried by a large transposing element and the $zeste^1$ allele in *Drosophila melanogaster.* Genetics **112:** 551–575.

GUBB, D., S. MCGILL and M. ASHBURNER, 1988 A selective screen to recover chromosome deletions and duplications in *Drosophila melanogaster.* Genetics **119:** 377–390.

GUBB, D., M. ASHBURNER, J. ROOTE and T. DAVIS, 1990 A novel transvection phenomenon affecting the *white* gene of *Drosophila melanogaster.* Genetics **126:** 167–176.

HAZELRIGG, T., R. LEVIS and G. M. RUBIN, 1984 Transformation of *white* locus DNA in *Drosophila:* dosage compensation, *zeste* interaction and position effects. Cell **36:** 469–481.

HENIKOFF, S., 1990 Position-effect variegation after 60 years. Trends Genet. **6:** 422–426.

HENIKOFF, S., 1994 A reconsideration of the mechanism of position effect. Genetics **138:** 1–5.

HENIKOFF, S., J. M. JACKSON and P. B. TALBERT, 1995 Distance and

pairing effects on the *brown*$^{[Dominant]}$ heterochromatic element in *Drosophila*. Genetics **140:** 1007–1017.

ISING, G. and K. BLOCK, 1981 Derivation dependent distribution of insertion sites for a *Drosophila* transposon. Cold Spring Harbor Symp. Quant. Biol. **45:** 527–544.

JACK, J. W. and B. H. JUDD, 1979 Allelic pairing and gene regulation: a model for the zeste-white interaction in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **76:** 1368–1372.

JUDD, B. J., 1995 Mutations of *zeste* that mediate transvection are recessive enhancers of position effect variegation in *Drosophila melanogaster*. Genetics **141:** 245–253.

KALISCH, W-E., and B. RASMUSON, 1974 Changes of zeste phenotype induced by autosomal mutations in *Drosophila melanogaster*. Hereditas **78:** 97–104.

KAUFMAN, T. C., S. E. TASAKA and D. SUZUKI, 1973 The interactions of two complex loci, zeste and bithorax in *Drosophila melanogaster*. Genetics **75:** 299–321.

LEISERSON, W. M., N. M. BONINI and S. BENZER, 1994 Transvection at the *eyes absent* gene of Drosophila. Genetics **138:** 1171–1179.

LEFEVRE, G., 1976 A photographic representation and interpretation of the polytene chromosomes of *Drosophila melanogaster* salivary glands, pp. 31–66 in *The Genetics and Biology of Drosophila*, Vol. 1a, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

LEVIS, R. W., P. M. BINGHAM and G. M. RUBIN, 1982 Physical map of the white locus of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **79:** 564–568.

LEWIS, E. B., 1954 The theory and application of a new method for detecting chromosomal rearrangements in *Drosophila melanogaster*. Am. Nat. **88:** 225–239.

LINDSLEY, D. L., and G. G. ZIMM, 1992 *The Genome of Drosophila melanogaster*. Academic Press, New York.

LINDSLEY, D. L., L. SANDLER, B. S. BAKER, A. T. C. CARPENTER, R. E. DENELL *et al.*, 1972 Segmental aneuploidy and the genetic gross structure of the *Drosophila* genome. Genetics **71:** 157–184.

LOVERING, R., 1988 A molecular analysis of *TE* mediated rearrangements in *Drosophila melanogaster*. Ph.D. Thesis University of Cambridge, UK.

LOVERING, R., N. HARDEN and M. ASHBURNER, 1991 The molecular structure of *TE146* and its derivatives in *Drosophila melanogaster*. Genetics **128:** 357–372.

LUCCHESI, J. C., 1973 Dosage compensation in *Drosophila*. Annu. Rev. Genet. **7:** 225–237.

PARO, R., M. L. GOLDBERG and W. J. GEHRING, 1983 Molecular analysis of a large transposable element carrying the white locus in *Drosophila melanogaster*. EMBO J. **2:** 853–860.

PERSSON, K., 1976 Modification of the eye color mutant *zeste* by Suppressor, Enhancer and Minute genes in *Drosophila melanogaster*. Hereditas **82:** 111–120.

PIRROTTA, V., 1991 The genetics and molecular biology of *zeste* in *Drosophila melanogaster*. Adv. Genet. **29:** 301–448.

PIRROTTA, V. and Ch. BROCKL, 1984 Transcription of the *Drosophila white* locus and some of its mutants. EMBO. J. **3:** 563–568.

PIRROTTA, V., H. STELLER and M. P. BOZZETTI, 1985 Multiple upstream regulatory elements control the expression of the *Drosophila white* gene. EMBO J. **4:** 3501–3508.

PIRROTTA, V., E. MANET, E. HARDON, S. E. BICKEL and M. BENSON, 1987 Structure and sequence of the *Drosophila zeste* gene. EMBO. J. **6:** 791–799.

POTTER, S., M. TRUETT, M. PHILLIPS and A. MAHER, 1980 Eukaryotic transposable elements with inverted terminal repeats. Cell **20:** 639–647.

ROOTE, J., D. GUBB and M. ASHBURNER, 1996 Aberrations in the *l(2)34Fc-l(2)35Bb* region of chromosome arm 2L. Drosophila Inf. Serv. **77:** 55–65.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning, A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SEECOF, R. L., W. D. KAPLAN and D. G. FUTCH, 1968 Dosage compensation for enzyme activities in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **62:** 528–535.

SMOLIK-UTLAUT, S. M. and W. M. GELBART, 1987 The effects of chromosomal rearrangements on the *zeste-white* interaction in *Drosophila melanogaster*. Genetics **116:** 285–298.

ZACHAR, Z. and P. M. BINGHAM, 1982 Regulation of white locus expression: the structure of mutant alleles at the white locus of *Drosophila melanogaster*. Cell **30:** 529–541.

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