The Drosophila *zeste* Locus Is Nonessential

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ABSTRACT

Diepoxybutane-induced mutations of the Drosophila *zeste* locus were generated in an effort to obtain a null allele. Of 33 mutations of this *X*-linked gene isolated, 16 were associated with multilocus deletions of *zeste* and adjacent complementation groups, while the remainder were defects restricted to *zeste* undetectable by Southern blot analysis. Two of these multilocus deletions ($Df(1)z^{deb3}$ and $Df(1)z^{deb92}$) were employed in the synthesis of females completely deleted for zeste. Such "zesteless" flies were produced, though at frequencies lower than Mendelian expectations. *zeste*-deleted females are fertile, and can give rise to *zeste*-deleted female progeny. These results demonstrate that the product of the *zeste* gene is not essential to viability or to female fertility, even if absent both as a maternal contribution and as a product of the zygotic genome. However, the possibility that *zeste* may influence relative viability cannot be excluded. In spite of previous *in vitro* indications that the *zeste* protein may activate transcription of the *Ultrabithorax* (*Ubx*) gene, *zeste* -deleted flies are Ubx^+ in phenotype. This suggests that the *zeste* protein normally is either a very weak transcription factor, or that its function can be substituted by that of other regulatory proteins.

THE *zeste* (*z*) locus of *Drosophila melanogaster* regulates the expression of at least three target genes: *white* (*w*), *decapentaplegic* (*dpp*), and the *Ultrabithorax* (*Ubx*) gene of the *bithorax* complex (BX-C). Activity of the *zeste* locus product is also intimately involved in the phenomenon of transvection, or pairing-dependent gene expression, which has been observed at each of these three target loci (LEWIS 1954; JACK and JUDD 1979; GELBART 1982; GELBART and WU 1982). Transvection effects are of considerable interest as their existence implies that regulatory signals can be transmitted in *trans* between the copies of a gene present on homologous chromosomes in a diploid organism.

The relationship between *zeste* and transvection phenomena is perhaps best illustrated through the interaction of the z^1 allele of *zeste* with the *white* (*w*) locus. The z^1 mutation yields aberrant yellow eye color in the presence of two paired copies of the w^+ gene, but if synapsis between chromosomal regions containing these w^+ genes is prevented, wild-type bright red eye color results (GANS 1953; JACK and JUDD 1979). This difference in pigmentation is associated with reduction in the accumulation of *white* mRNA molecules in the heads of yellow-eyed flies (BINGHAM and ZACHAR 1985). Furthermore, reactivity of paired *white* genes to z^1 depends upon sequences located upstream of the start of *white* transcription (GREEN 1959; DAVISON *et al.* 1985; LEVIS, HAZELRIGG and RUBIN 1985; PIRROTTA, STELLAR and BOZETTI 1985). Recent experiments have shown that the protein encoded by the *zeste* locus specifically binds to these upstream sequences at *white* (BENSON and PIRROTTA 1987; MANSUKHANI *et al.* 1988b). Presumably, binding of the *zeste* product (or of the mutant z^1 product) modifies the level of *white* transcription. At least *in vitro*, addition of wild-type *zeste* protein increases the rate of transcription from the *Ubx* promoter (BIGGIN *et al.* 1988).

The null state of *zeste*: Further attempts to understand the normal function of the *zeste* gene product have been hampered by uncertainty concerning the null state of *zeste*. In addition to z^1, several different kinds of mutations at *zeste* have been described. The genetic behavior of the z^a group of alleles displays properties expected for a null mutation (KAUFMAN, TASAKA and SUZUKI 1973). For example, both z^a lesions and deficiencies for the region containing *zeste* are recessive to z^+ and to z^1. Thus, in an otherwise normal background, z^a/z^+ and $Df(z)/z^+$ flies have wild-type red eyes, while z^a/z^1 and $Df(z)/Z^1$ animals have yellow eyes.

Other aspects of z^a-like mutations are more difficult to reconcile with the view that they are completely deficient for *zeste* activity. For example, though *in vitro* experiments indicate that *zeste* protein may serve as a transcriptional activator of *Ubx* (BIGGIN *et al.* 1988), z^a mutations have no obvious phenotypic effects on expression of the wild-type *bithorax* complex (KAUFMAN, TASAKA and SUZUKI 1973). In a similar vein, homozygosity or hemizygosity for z^a yields wild-

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type, instead of mutant, eye color. Finally, recent molecular analyses suggest that z^a mutations could encode products which potentially retain some activity as a transcriptional regulator (GUNARATNE *et al.* 1986; PIRROTTA *et al.* 1987; MANSUKHANI *et al.* 1988a, b). A domain of the *zeste* protein near the N terminus is necessary and sufficient for its site-specific DNA binding properties; no currently characterized *zeste* mutation would result in alteration of this domain. An adjacent acidic region of the protein, which might have transcriptional activation function, is similarly unaffected by these mutations (MANSUKHANI *et al.* 1988a, b).

As it is unclear whether z^a or other known alleles characterize the null state of *zeste*, it has been difficult to determine the *in vivo* role of its product. For example, *zeste* protein activity might be required for viability or fertility. Whether or not absence (as opposed to a mutant form) of the *zeste* product might influence the expression of identified target genes such as *white* and *Ubx* has similarly been in question. In this paper, we report experiments designed to generate Drosophila completely deficient for the *zeste* locus. Our results indicate that the *zeste* product is nonessential for viability or female fertility. Flies deleted for *zeste* are Ubx^+ in phenotype, in spite of its *in vitro* activity as a transcriptional enhancer of *Ubx* expression.

MATERIALS AND METHODS

Drosophila culture conditions and stocks: Unless indicated otherwise, Drosophila cultures were raised at 22° on standard yeast glucose medium (ASHBURNER and THOMPSON 1978).

Visible markers: The *cv f* and *y z^1 spl sn* stocks used in the mutagenesis were from the collection of ROSS MACINTYRE, Cornell University, Ithaca, New York. The strain z^1 $Dp(1;1)w^{+R}$, containing a tandem duplication of approximately 80 kb of DNA containing the proximal part of the *white* locus, was the gift of M. M. GREEN, University of California, Davis. Because of the duplication, both males and females of this strain display the z^1 eye color (GREEN 1963; GOLDBERG *et al.* 1983). Descriptions of all other visible mutations can be found in LINDSLEY and GRELL (1968).

Deficiencies: The previously characterized chromosome deficiencies $Df(1)w^{rJ1}$ (breakpoints 3A3,4; 3C1,3) and *Df(1)64c4* (breakpoints 3A3,4; 3C3,5) are described in LINDSLEY and GRELL (1968), and were obtained from BURKE JUDD, NIEHS, Research Triangle Park, North Carolina.

Lethals in the zeste-white region: Alleles of most lethal complementation groups in the *zeste*-white region (JUDD, SHEN and KAUFMAN 1972) were also obtained from B. JUDD. *l(1)zw13* was provided by LEONARD ROBBINS, Michigan State University, East Lansing; and $l(1)gt^{X11}$ was from MARIANA WOLFNER, Cornell University, Ithaca, New York.

Y chromosome derivatives: The *Y* chromosome derivatives $w^+ \cdot Y$ and $y^+w^+B^S \cdot Y$, both of which carry duplications of the *zeste*-white interval, are described in LINDLEY and GRELL (1968), and were obtained from B. JUDD and M. M. GREEN, respectively.

Balancer chromosomes: *FM7a* = $In(1)sc^8$ + *15D-E; 20A-E* + *dl-49*, y^{31d} sc^8 w^a v^0 *B* (MERRIAM 1968).

CyO = *In(2LR)O, Cy* dp^{lvl} cl^4 *pr* cn^2 (KOTARSKI, PICKERT and MACINTYRE 1983).

Gla = *In(2LR)Gla.*

TM3 = *In(3LR)* y^+ *ri* p^p *sep* bx^{34e} e^s, *Sb Ser.*

Complete descriptions of balancer chromosomes are found in LINDSLEY and GRELL (1968) and LINDSLEY and ZIMM (1987). All balancers were obtained from R. MACINTYRE.

Transposons: Stocks carrying the second chromosome transposon insertions $P[ry^+,tko^+]_{22.1}$ and $P[ry^+,tko^+]_{236.2}$ were obtained from C. ROYDEN, University of California, San Francisco, and are described in ROYDEN, PIRROTTA and JAN (1987). A strain carrying the transposon $P[z^+]_{B1}$, a 5.8-kb *Eco*RI fragment encompassing the *zeste* gene inserted into region 63C,D of chromosome *3*, was previously described (GUNARATNE *et al.* 1986).

Isolation of diepoxybutane-induced *zeste* mutations: *cv f/Y* males less than 2 days old were fed the chemical mutagen diepoxybutane (DEB; Sigma) at a concentration of 10 mM in a 1% sucrose solution for 24 hr, according to the protocol of CROSBY and MEYEROWITZ (1986). Treated males were mated with *y z^1 spl sn* virgin females; 30 males and 30 females were added to each bottle, which were brooded 3 times at 3-day intervals. Progeny without lesions induced at *zeste* include red-eyed *y z^1 spl sn/Y* males and red-eyed *y z^1 spl sn/*(z^+) *cv f* females. Female progeny showing some yellow pigmentation in either or both eyes were selected as potential *zeste* null mutants, as $z^1/Df(1)z$ females are yellow-eyed.

Females with potential novel *zeste* mutations were mated with *y z^1 spl sn* males to resolve mosaics. Of the 96 strains identified, only 33 were found to transmit the aberrant eye color. All of these 33 mutations were independent, with the possible exception of $Df(1)z^{deb92}$ and $Df(1)z^{deb910}$. Yellow-eyed y^+ spl^+ females of the second generation were then crossed to *FM7a/Y* males to establish stocks; strains with male viable *zeste* mutations were later preserved as homozygotes.

Complementation analysis: Each male inviable novel *zeste* mutation (z^{deb}*) was tested by complementation crosses to establish whether lethal loci adjacent to *zeste* ($l(1)zw_x$) were also affected. Females of genotype z^{deb}*/*FM7a* were crossed with $l(1)zw_x/w^+ \cdot Y$ males in vials. The absence of any Bar^+ females in more than 50 progeny was used as the criterion for failure to complement the lethal gene tested. The absence of $z^{deb}*/w^+ \cdot Y$ males among the progeny suggests that z^{deb}* is either a deletion extending beyond the borders of the $w^+ \cdot Y$ translocation, or that the *X* chromosome carries a second lethal hit outside of the *zeste* region. These possibilities could be distinguished by subsequent crosses of z^{deb}*/*FM7a* females to $Df(1)w^{rJ1}/y^+w^+B^S \cdot Y$ males; the eclosion of $z^{deb}*/Df(1)w^{rJ1}$ female progeny indicates that no essential genes near *zeste* on the z^{deb}* chromosome are defective.

Cytology: Temporary larval salivary gland squashes were made by dissecting the glands of $z^{deb}*/w^+ \cdot Y$ males or $z^{deb}*/z^{deb}*$ females according to the method of KOTARSKI, PICKERT and MACINTYRE (1983). Prior to squashing, a drop of 2% orcein-0.25% carmine stain in 50% propionic acid was added to the dissected gland. The cytology of mutants was compared to that of *cv f* parental males and to the map of LEFEVRE (1976).

Synthesis of *zeste*-deleted females: The cross scheme used for the construction of flies deficient for the *zeste* locus in outlined on Figure 1. Starting material included: *FM7a/FM7a* females; *CyO/Gla* (second chromosome balancers used to track the $P[ry^+,tko^+]$ transposons) males; $P[ry^+,tko^+]_{22.1}$/*CyO* and $P[ry^+,tko^+]_{236.2}$/*CyO* males and females; z^{deb3}/*FM7a* and z^{deb92}/*FM7a* females; and $Df(1)w^{rJ1}/y^+w^+B^S \cdot Y$ males. In-

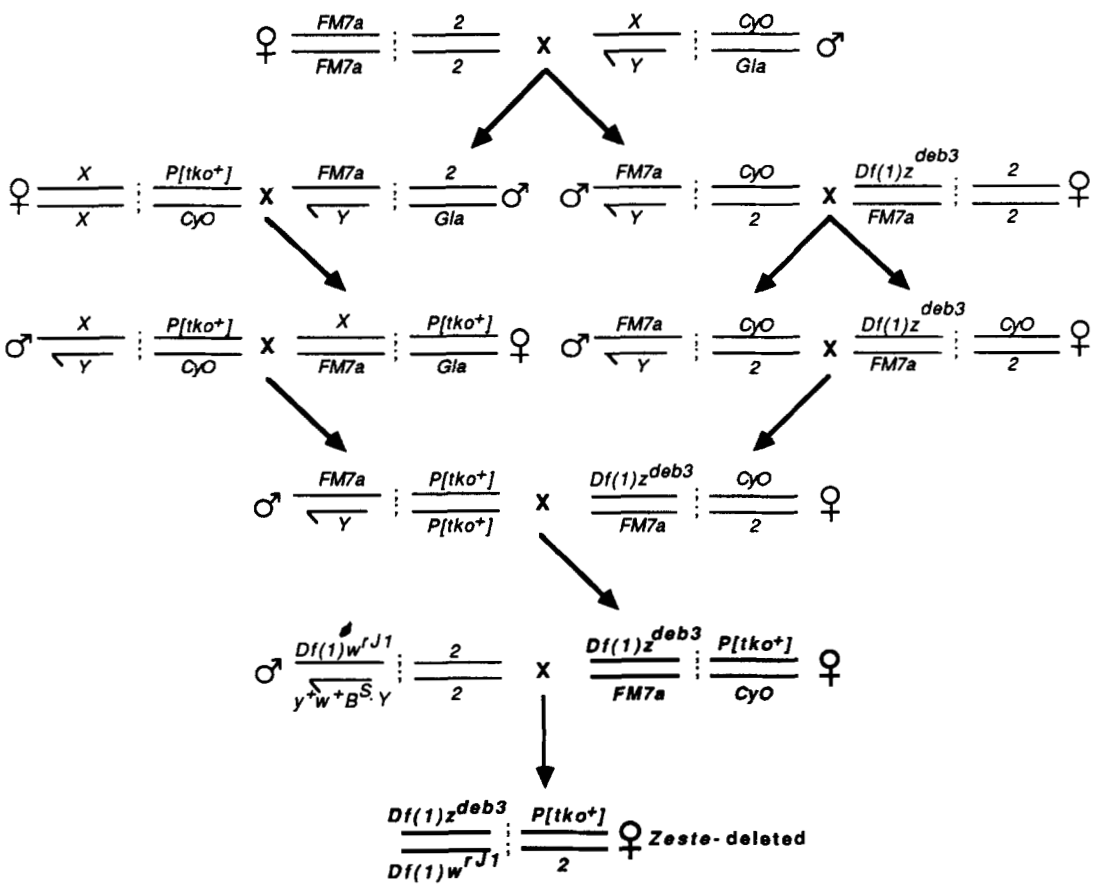

FIGURE 1.—Cross scheme for synthesis of *zeste*-deleted females. $Df(1)z^{deb3}/FM7a$; $P[ry^+,tko^+]/CyO$ mothers of *zeste*-deleted females, as well as $Df(1)z^{deb3}/Df(1)w^{rJ1}$; $P[ry^+,tko^+]/2$ animals lacking *zeste* are shown in bold type for reference.

dependent experiments using the two z^{deb} deletions and the two $P[ry^+,tko^+]$ transposons in all possible combinations were performed to minimize the chance that strain-specific effects would be observed.

Successful construction of $z^{deb3\text{ or }92}/FM7a$; $P[ry^+,tko^+]/CyO$ females (bold type in Figure 1) was checked by mating these flies with $tko/w^+ \cdot Y$ males. Bar$^+$ female progeny were produced, all of which were Cy$^+$, indicating that the unmarked second chromosome indeed contained the tko^+ transposon.

zeste-deleted females were synthesized both as the progeny of $z^{deb3\text{ or }92}/FM7a$; $P[ry^+,tko^+]/CyO$ females (bold type in Fig 1), and as the progeny of $z^{deb3\text{ or }92}/FM7a$; $P[ry^+,tko^+]/P[ry^+,tko^+]$ females in subsequent crosses (not diagrammed). Both sets of data for all four combinations of the two $P[ry^+,tko^+]$ transposons and the two z^{deb} deficiencies tested gave equivalent and consistent results.

Addition of a *zeste* transposon to *zeste*-deleted females: A mixture of phenotypically indistinguishable $Df(1/w^{rJ1}/y^+w^+B^S \cdot Y$; $P[z^+]_{B1}/3$ and $Df(1)w^{rJ1}/y^+w^+B^S \cdot Y;3/3$ males was generated from the cross scheme depicted in Figure 2. Individual males were tested for the presence of the z^+ transposon by crossing to z^1 $Dp(1;1)w^{+R}$ females; as expected, one-half of these males (those containing $P[z^+]_{B1}$) gave rise to red-eyed female progeny ($Df(1)w^{rJ1}/z^1$ $Dp(1;1)w^{+R}$; $P[z^+]_{B1}/2$; data not shown). This mixture of males was also mated with $z^{deb3\text{ or }92}/FM7a$; $P[ry^+,tko^+]/P[ry^+,tko^+]$ females (see RESULTS). As an additional check, the same cross was repeated with individual males. Because of their low viability, *zeste*-depleted females emerged from only 8/37 crosses, rendering analysis difficult. However, it was apparent that the viability of bright red-eyed *zeste*-deleted progeny was not improved relative to $Df(1)w^{rJ1}/FM7a$ siblings by addition of the z^+ transposon (see DISCUSSION).

Nucleic acid procedures: The purification of genomic DNA from adult Drosophila, digestion with restriction enzymes, transfer to GeneScreen filters (New England Nuclear), labeling of nucleic acids, and the hybridization of labeled probes to filter blots have been previously described (GUNARATNE *et al.* 1986).

RESULTS

Mutagenesis: To generate a mutation which could in theory be unambiguously identified at the DNA level as a null allele of *zeste*, we chose the mutagen DEB. This agent was previously described to induce the formation of small deletions observable by whole genome Southern analysis (SHUKLA and AUERBACH 1980; OLSEN and GREEN 1982; CROSBY and MEYEROWITZ 1986; REARDON *et al.* 1987). Limiting the size of resultant deficiencies was also an important consideration, as the *zeste* gene lies very close to the adjacent lethal complementation group *tko*. Only deletions restricted to *zeste* would yield interpretable results.

For the mutagenesis, *cv f* males carrying the wild-type allele of *zeste* were treated with DEB, and subsequently mated with *y* z^1 *spl sn* homozygous females. As z^+ is normally dominant to z^1, the majority of

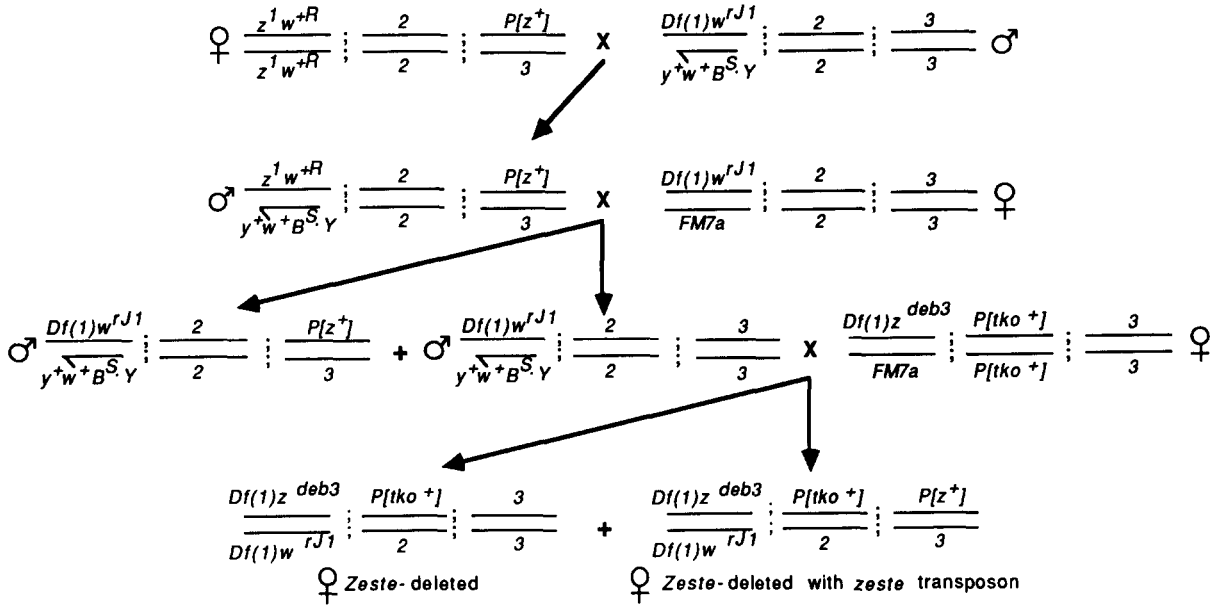

FIGURE 2.—Cross scheme for addition of the *zeste*$^+$-containing transposon $P[z^+]_{B1}$ to *zeste*-deleted females. $Df(1)z^{deb3}/FM7a$; $P[tko^+]/P[tko^+]$; *3/3* females used in the final mating were derived from the series of crosses depicted in Figure 1. This last mating was performed both (1) with a mixture of males either containing or not containing $P[z^+]_{B1}$ (data presented in Table 4) or (2) with individual males (see MATERIALS AND METHODS).

female progeny (F_1) will have red eyes. If these daughters received a chromosome with an induced deletion of *zeste*, they will display aberrant yellow eye color. After an additional round of mating with *y z^1 spl sn* males to resolve somatic or germline mosaics, mutagenized chromosomes were placed into stock. The flanking markers *y*$^+$ and *spl*$^+$, closely linked to *zeste*, tagged the mutagenized chromosome through this procedure.

Ninety-six of approximately 300,000 F_1 females displayed some yellow eye pigmentation, partially or completely covering one or both eyes. Thirty-three of these females transmitted a mutation of *zeste* to subsequent generations. These frequencies are similar to those experienced by other investigators using the mutagen DEB (SHUKLA and AUERBACH 1980; OLSEN and GREEN 1982; CROSBY and MEYEROWITZ 1986; REARDON *et al.* 1987).

Male-viable mutations: Of the 33 *zeste* alleles obtained, 17 were viable both in hemizygous males and homozygous females. Hemizygous males from 16 of these lines were fertile, so homozygous stocks could be established. The eye color in both sexes of the homozygous stocks was wild-type or near wild-type. Males from the additional mutant strain were infertile, and were maintained in stock with the *X* chromosome balancer FM7a. The male sterility of this line is unlikely to result from lesions of *zeste*, as infertility could not be rescued by *X;Y* translocations such as *w*$^+$·*Y*, which contain the *z*$^+$ gene.

Certain differences in the phenotypes of flies heterozygous for the *z*^{1} mutation and the treated chromosome (*z*^{1}/*z*^{deb}*) were observed. Some of these heterozygotes displayed homogeneous bright yellow *zeste* eye pigmentation, while scattered ommatidia in the remainder were red. These uneven patterns are similar in appearance to those seen in adults carrying *white-spotted* (*w*^{sp}) mutations, which disrupt sites at the *white* locus to which the *zeste* product can bind. We interpret the homogeneous eye color as a manifestation of the *z*^{1} allele in *z*^{deb}* backgrounds with little or no *zeste* expression, while the variegated patterns result from weakly hypomorphic *z*^{deb}* mutations.

Polytene chromosomes in several of these male-viable strains appear normal near the cytogenetic location of *zeste*. Preliminary analysis by whole genome Southern blotting has revealed no obvious alterations in restriction enzyme fragments from the *zeste* locus region in any male-viable line (data not shown). These blots could resolve differences in fragment length of approximately 100 bp in the appropriate region; more refined experiments are thus required to determine whether some or all of these male-viable mutations are associated with smaller deletions in or near *zeste*.

Male-inviable mutations: Of the 33 mutant *X* chromosomes recovered from the screen, the remaining 16 were lethal in males. The lethal lesions in two of these chromosomes do not map to the interval containing *zeste*, as females heterozygous for these mutations and a deficiency including *zeste* ($Df(1)w^{rJ1}$) are viable. These strains thus contain defects both at *zeste* and at sites elsewhere on the *X* chromosome. The mutations in the vicinity of *zeste* were not associated with alterations visible on whole genome Southern

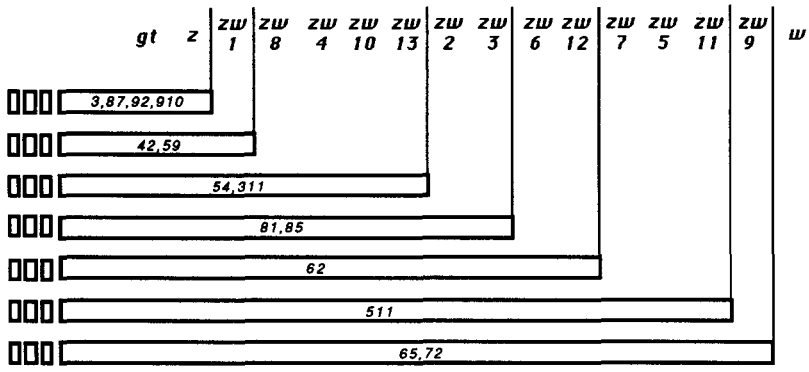

FIGURE 3.—Extent of DEB-induced multilocus deficiencies. Complementation groups named at the top are deleted when they lie within the open bars. Numbers within the bars denote particular DEB-induced deletions. All of these rearrangements remove the gene *l(1)gt*; the distal (leftmost) limit of the deletions have not been determined.

blots; these two strains will not be further considered here.

Complementation analysis shows that all the 14 remaining male lethal mutations are associated with deficiencies including *zeste* and adjacent complementation groups (Figure 3). This collection includes deletions of varying extents. The centromere proximal (right) borders of these deletions are found at several locations in the interval between *zeste* and *white*. The centromere distal (left) borders have not been mapped, though all of these deficiencies remove the locus *l(1)gt*. Examination of polytene chromosomes reveals associated physical deletions in those stocks analyzed. Relevant to the studies discussed below, bands from 2E,F to 3A3 are missing in $Df(1)z^{deb3}$ and $Df(1)z^{deb92}$ chromosomes.

Strategy for construction of a *zeste* deficiency: None of the 33 mutations recovered in the screen has yet been demonstrated to be a defect restricted to *zeste* which completely abolishes function of this gene. The male-inviable deficiencies are not useful for interpretation of the null phenotype of *zeste* because adjacent loci are affected, while the lesions in the male-viable group are subtle and have not yet been characterized. However, two of the deficiencies ($Df(1)z^{deb3}$ and $Df(1)z^{deb92}$; see Figure 3) could be employed in the synthesis of flies devoid of *zeste*-coding sequences. Combination of either deletion with the well-characterized rearrangement $Df(1)w^{rJ1}$ (*i.e.*, $Df(1)z^{deb3}/Df(1)w^{rJ1}$) would result in the absence of only two known genes: *zeste* and the adjacent lethal complementation group *tko*. The lesion for *tko* could be corrected by an autosomal copy of *tko* but not *zeste* (Figure 4); the integration of appropriate *tko* sequences into autosomes by *P* element-mediated transformation has previously been reported (ROYDEN, PIRROTTA and JAN 1987).

This approach requires that $Df(1)z^{deb3}$ or $Df(1)z^{deb92}$ should be incapable of encoding functional *zeste* product. Blots of restriction enzyme-cut $Df(1)z^{deb3}/FM7a$ and $Df(1)z^{deb92}/FM7a$ genomic DNAs with probes in the vicinity of *zeste* reveals only bands from the *FM7a* balancer chromosome (not shown). Our data indicates that both DEB-induced deficiencies remove the entire *zeste* gene and at least 30 kb of sequences further in the centromere-proximal (right) direction. The physical extent of these and other deficiencies as well as the transposons used in our experiments are shown in Figure 5. As subsequent experience with $Df(1)z^{deb3}$ and $Df(1)z^{deb92}$ proved identical, only the former will be discussed for simplicity.

It was formally possible that this span of 30 kb or more proximal to *zeste* contains an essential gene or genes, not identified in previous saturation mutagenesis of the region, which would complicate the analysis. We thus performed the pilot cross shown in Table 1, to establish whether females of genotype $Df(1)z^{deb3}/Df(1)64c4$ are produced at expected rates. Such heterozygous females are in fact viable, indicating the absence of novel lethal complementation groups in the region of overlap to the right of *zeste* between these two deletions. This overlap is identical to that produced by combination of $Df(1)z^{deb3}$ and $Df(1)w^{rJ1}$, with the exception of about 10 kb including the majority of the *zeste* and *tko* genes (Figure 5). The strategy outlined in Figure 4 should thus serve to produce animals deleted only for *zeste*.

Properties of *zeste*-deleted females: The scheme employed for the desired construction is diagrammed in Figure 1. Females of the phenotype anticipated for the combination of chromosomes $Df(1)z^{deb3}/Df(1)w^{rJ1}$; $P[ry^+,tko^+]/2$ were produced (Table 2). Several observations indicate that these animals are indeed *zeste*-deleted females. First, their dull reddish-brown eye color (in contrast to the bright red wild-type eye pigmentation) suggests a lesion at *zeste*. This eye color appears to be characteristic of females carrying one copy of a z^a-like *zeste* allele and one copy of w^+ ($Df(1)w^{rJ1}$ deletes w^+); similar, if not identical, phenotypes are seen in $Df(1)w^{deb3}/Df(1)64c4$ (see Table 1) and $z^a/Df(1)w^{rJ1}$ females. Second, the appearance of these flies is dependent upon the presence of the transposon containing tko^+. None of over 200 "zesteless" progeny of mothers heterozygous for the tko^+ transposon and a marked balancer chromosome (Figure 1) contained the balancer chromosome (data not

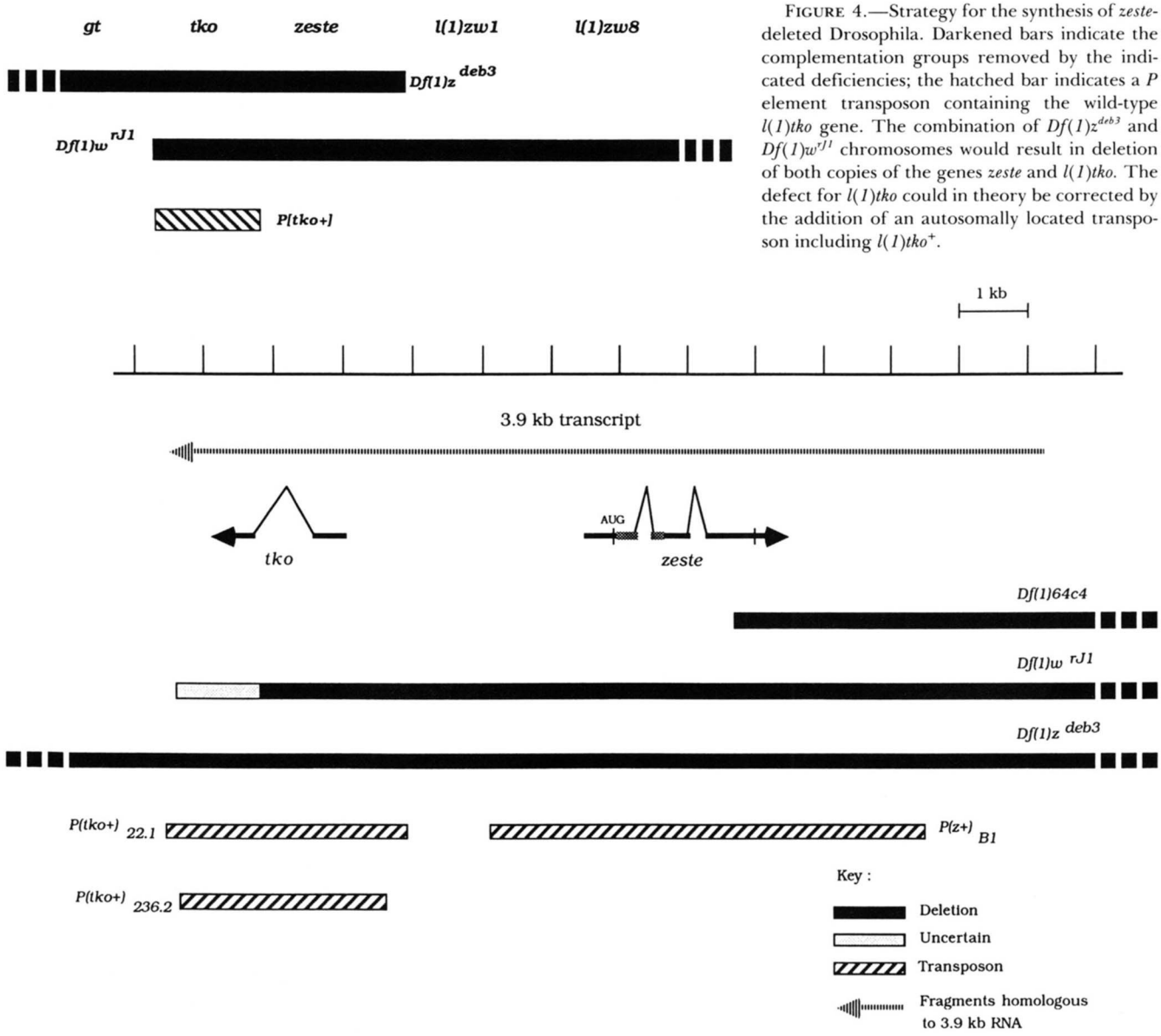

FIGURE 4.—Strategy for the synthesis of *zeste*-deleted Drosophila. Darkened bars indicate the complementation groups removed by the indicated deficiencies; the hatched bar indicates a *P* element transposon containing the wild-type *l(1)tko* gene. The combination of $Df(1)z^{deb3}$ and $Df(1)w^{rJ1}$ chromosomes would result in deletion of both copies of the genes *zeste* and *l(1)tko*. The defect for *l(1)tko* could in theory be corrected by the addition of an autosomally located transposon including $l(1)tko^{+}$.

FIGURE 5.—Physical map of the region of the Drosophila *X* chromosome in the vicinity of *zeste*. Data has been collected from GUNARATNE *et al.* (1986), PIRROTTA *et al.* (1987), ROYDEN, PIRROTTA and JAN (1987) and MANSUKHANI *et al.* (1988a). The telomere-to-centromere (distal-to-proximal) direction proceeds from left to right on the diagram. DNA fragments with homology to a 3.9-kb transcript of unknown function are denoted by the hatched arrow; the organization of introns and exons of the corresponding transcriptional unit is not known. Transcripts for *zeste* and *l(1)tko* are indicated by darkened arrows, with introns shown by normal conventions. Sequences encoding the DNA binding domain of the *zeste* protein as defined by MANSUKHANI *et al.* (1988b) are portrayed by stippled regions within the *zeste* mRNA. The extent of deficiencies and transposons used in this study are indicated as shown in the key.

shown). Third, the kinds of progeny produced by the *zeste*-deleted females are exactly as predicted (Table 3). Finally, *zeste* gene sequences are absent in genomic DNA prepared from $Df(1)z^{deb3}/Df(1)w^{rJ1}$; $P[ry^{+},tko^{+}]/2$ animals when analyzed on Southern blots (Figure 6).

Apart from slightly abnormal eye color, *zeste*-deleted females appear morphologically normal. These flies do not obviously display the transformation of haltere into wing associated with mutations of *Ubx*, though subtle defects cannot be excluded. Females without a *zeste* locus are fertile, and are of normal or near-normal fecundity. When appropriate matings are conducted, they yield a subsequent generation of *zeste*-deleted females (Table 3).

Table 2 indicates that $Df(1)z^{deb3}/Df(1)w^{rJ1}$; $P[ry^{+}, tko^{+}]/2$ adults are produced at frequencies much lower than expected. These *zeste*-deleted flies emerge at a frequency only 5 to 10% of that for $Df(1)w^{rJ1}/FM7a$ females, the alternative products of the same maternal disjunction events. "Zesteless" females similarly comprise a relatively rare class of the progeny of *zeste*-deleted mothers (Table 3). The addition of a transposon containing the wild-type *zeste* gene to *zeste*-deleted females does not correct the lowered viability of these flies, even though this transposon rescues their aber-

TABLE 1

The region near *zeste* contains no unidentified lethal complementation groups

Progeny class	Cross A		Cross B	
	Number	Percent	Number	Percent
$Df(1)z^{deb^*}/Df(1)64c4$ ♀[a]	241	30.8	218	29.0
$FM7a/Df(1)64c4$ ♀	118	15.1	77	10.3
$Df(1)z^{deb^*}/w^+ \cdot Y$ ♂	290	37.1	197	26.2
$FM7a/w^+ \cdot Y$ ♂	133	17.0	259	34.5
Total	782		751	

Cross A = $Df(1)z^{deb3}/FM7a$ ♀ X $Df(1)64c4/w^+ \cdot Y$ ♂; cross B = $Df(1)z^{deb92}/FM7a$ ♀ X $Df(1)64c4/w^+ \cdot Y$ ♂.

[a] Eye color phenotype is dull red. Addition of $P[z^+]_{B1}$ to this genotype results in bright red wild-type eye pigmentation (not shown).

rant dull reddish-brown eye color to wild-type (Table 4). This result suggests, but does not prove, that the relative inviability is not a function of the defect at *zeste* (see below).

DISCUSSION

Mutagenesis: Investigators using the reagent diepoxybutane as a mutagen for Drosophila have reported somewhat conflicting results. In the case most thoroughly studied at the molecular level, of 21 DEB-induced mutations at the *rosy* locus, 9 were caused by deletions ranging in size from 50 bp to 8 kb, 7 were caused by lesions too small to be detected by Southern blot analysis, and only 2 were associated with large multilocus deletions (REARDON *et al.* 1987). In another study, all 8 DEB-induced mutations in the 68A3-68C11 region uncovered only a single complementation group (CROSBY and MEYEROWITZ 1986). Other workers have found that large multilocus deficiencies comprise a much higher percentage (30–60%) of DEB-induced mutations at the *y*, *w*, *sn*, and *m* loci (SHUKLA and AUERBACH 1980; OLSEN and GREEN 1982). Our experience more closely parallels these latter studies. We have isolated two large classes of DEB-induced mutations at *zeste*: (1) male viable *zeste* alleles associated with nucleotide substitutions or deletions below the level of detection (<100 bp), and (2) deletions of *zeste* and adjacent genes. Based upon known physical data, the shortest of these male-inviable deletions must be greater than 30 kb in length, while the longest extend over 200 kb.

We do not understand these discrepancies, particularly the lack of deletions in the range of 50 bp to 8 kb in our study, as well as the variability in the frequency of longer deletions obtained by different investigators. These disagreements might be related to peculiarities of DNA sequence in various regions. In this regard, we are currently testing the hypothesis that instability of or recombination between transposable elements near *zeste* may be involved in the generation of the large, male inviable deletions. Such events might be independent of the application of DEB. Unequal crossing over between transposable elements on homologous chromosomes has been shown to generate deficiencies and reciprocal duplications during meiosis in Drosophila females (GOLDBERG *et al.* 1983), and intrachromosomal interactions between *hobo* elements have been shown to yield instability (LIM 1988). If similar recombinational mechanisms created the large deletions near *zeste*, these events must have occurred as intrachromosomal or interchromatid exchanges on the *X* chromosome in mutagenized *cv f* males.

Reduced yield of *zeste*-deleted females: Adult *zeste*-deleted females appear at frequencies of 5–10% of expectation (Tables 3 and 4). Changing the temperature at which the flies are raised to 18° or to 25° does not affect the yield of these adults (data not shown). The developmental stage of lethality is likely to be multiphasic. $Df(1)z^{deb3}/Df(1)w^{rJ1}$; $P[ry^+,tko^+]/2$ animals produced in the crosses diagrammed in Figure 1 are easily recognized as late pupae, as eye color and morphology is apparent at this stage, and because they are the only red-eyed, non-*Bar* of *Stone* (B^S) progeny produced in the crosses. Approximately 75–80% of these pupae never eclose (compared with <20% of other genotypes). Assuming random distribution of gametes, this suggests that 20–40% of the individuals which do not survive to adulthood die as late pupae, while the remainder die at earlier stages.

We have no satisfactory explanation for the relative inviability of *zeste*-deleted females; four possibilities are suggested here.

1. As described above, the addition of a transposon containing the wild-type *zeste* gene to *zeste*-deleted females does not improve their viability (Table 4). It is possible that the transposon employed is defective in some aspect of *zeste* expression, even though it rescues the *zeste* eye color phenotype (GUNARATNE *et al.* 1986; this article). For example, position effects at the site of $P[zeste^+]_{B1}$ transposon integration might disrupt transcription of *zeste* in particular tissues. Quantitative influences of *zeste* activity on viability thus cannot be excluded.

2. A similar argument also applies to the tko^+ transposons used in the construction of *zeste*-deleted females. These DNA segments complement the lethal point mutation tko^{k11} (ROYDEN, PIRROTTA and JAN 1987). This allele may exhibit some residual *tko* activity; the transposons might therefore be incapable of correcting the possibly more severe *tko* defect in $Df(1)w^{rJ1}$. However, as two different $P[ry^+,tko^+]$ transformants were employed, position effects are unlikely to have interfered with *tko* expression.

3. Figure 5 illustrates that $Df(1)z^{deb3}$ and $Df(1)w^{rJ1}$ remove sequences on both sides of the *zeste* gene which

TABLE 2

Yield of *zeste*-deleted females from representative crosses

Progeny class	Cross A		Cross B	
	Number	Percent	Number	Percent
$Df(1)z^{deb^*}/Df(1)w^{rJ1}$; $P[tko^+]/2$ (*zeste*-deleted) ♀[a]	15	1.2	20	3.3
$FM7a/Df(1)w^{rJ1}$; $P[tko^+]/2$ ♀	345	27.3	236	38.7
$Df(1)z^{deb^*}/y^+w^+B^s\cdot Y$; $P[tko^+]/2$ ♂	423	33.5	171	28.0
$FM7a/y^+w^+B^s\cdot Y$; $P[tko^+]/2$ ♂	360	28.5	135	22.1
$Df(1)z^{deb^*}/FM7a/y^+w^+B^s\cdot Y$; $P[tko^+]/2$ ♀[b]	121	9.6	48	7.9
Total	1264		610	

Cross A = $Df(1)z^{deb3}/FM7a$; $P[tko^+]_{22.1}/P[tko^+]_{22.1}$ ♀ × $Df(1)w^{rJ1}/y^+w^+B^s\cdot Y$ ♂; cross B = $Df(1)z^{deb92}/FM7a$; $P[tko^+]_{236.2}/P[tko^+]_{236.2}$ ♀ × $Df(1)w^{rJ1}/y^+w^+B^s\cdot Y$ ♂.

[a] Dull red eye color phenotype.

[b] Based upon additional evidence (not shown), this class appears to be the result of maternal nondisjunction, presumably due to the presence of an unmarked *Y* chromosome in some maternal genomes.

TABLE 3

Synthesis of second-generation *zeste*-deleted females

Progeny class	Number	Percent
$Df(1)z^{deb3}/Df(1)w^{rJ1}$; $P[tko^+]_{22.1}/2$ ♀ (*zeste*-deleted)	4	3.3
$Df(1)z^{deb3}$ *or* $Df(1)w^{rJ1}/y^+w^+B^s\cdot Y$ ♂[a]	118	95.9
$Df(1)z^{deb3}$ *or* $Df(1)w^{rJ1}/Df(1)w^{rJ1}/y^+w^+B^s\cdot Y$ ♀[a,b]	1	0.8
Total	123	

This table presents results of the following cross: $Df(1)z^{deb3}/Df(1)w^{rJ1}$; $P[tko^+]_{22.1}/2$ ♀ × $Df(1)w^{rJ1}/y^+w^+B^s\cdot Y$ ♂. Note that under Mendelian expectations (ignoring products of nondisjunction), *zeste*-deleted females should represent approximately 16% of total progeny.

[a] Due to the markers on the $y^+w^+B^s\cdot Y$ chromosome and recombination between maternal *X* chromosomes, genotypes containing $Df(1)z^{deb3}$ and $Df(1)w^{rJ1}$ cannot be distinguished. Individuals in these classes may or may not contain one copy of the $P[tko^+]_{22.1}$ transposon.

[b] Product of presumed maternal nondisjunction.

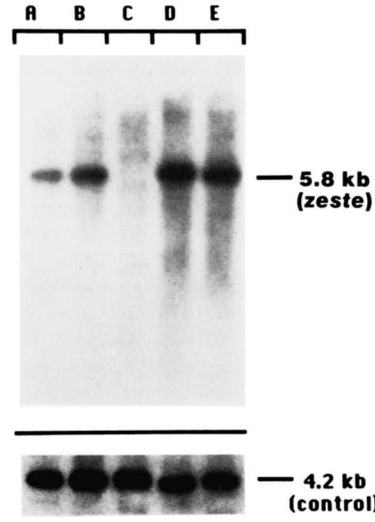

FIGURE 6.—Genomic Southern blots. (A) *FM7a* ♂; (B) $Df(1)z^{deb92}/FM7a$; $P[ry^+,tko^+]/CyO$ ♀ (mothers of *zeste*-deleted females; see Figure 1); (C) $Df(1)z^{deb3}/Df(1)w^{rJ1}$; $P[ry^+,tko^+]/2$ ♀ (*zeste*-deleted); (D) $Df(1)w^{rJ1}/y^+w^+B^S\cdot Y$ ♂ (fathers of *zeste*-deleted females; see Figure 1); (E) *cv f* ♀ (chromosome initially used to generate $Df(1)z^{deb92}$). DNA from 30 adults of each genotype (approximately 4 μg) was prepared and digested with the restriction enzyme *Eco*RI. After electrophoresis and transfer to GeneScreen Plus filters, the blot was probed with the corresponding cloned 5.8-kb *zeste* fragment from the Canton S wild-type strain (the same fragment was contained in the $P[z^+]_{B1}$ transposon shown in Figure 5). Results of this hybridization are shown in the top panel. Signals not in the 5.8-kb band represent genomic fragments with homology to sequences encoding tracts of polyglutamine and polyalanine within the *zeste* gene (PIRROTTA *et al.* 1987; MANSUKHANI *et al.* 1988a). In the bottom panel, the identical filter was reprobed with a fragment from the vicinity of *l(1)zw2* (J. MONTGOMERY, B. WILLIAMS and M. L. GOLDBERG, unpublished data), as a control for DNA loading.

encode a 3.9-kb poly(A$^+$) transcript (GUNARATNE *et al.* 1986; MANSUKHANI *et al.* 1988a). This transcriptional unit is not associated with any known genetic functions, but an effect on viability remains possible. However, it should be remembered that $Df(1)z^{deb3}/Df(1)64c4$ females emerge at expected frequencies, even though $Df(1)64c4$ removes sequences encoding the 5′ end of this 3.9-kb transcript (Figure 5).

4. Heterozygosity for two different deletions ($Df(1)z^{deb3}$ and $Df(1)w^{rJ1}$) may result in genetic imbalance and thus a decrease in survival of *zeste*-deleted females. This possibility is unlikely given that $Df(1)64c4$ appears to extend further in the centromere proximal direction (to bands 3C3-5) than does $Df(1)w^{rJ1}$ (to bands 3C1-3) (LINDSLEY and GRELL 1968), even though $Df(1)z^{deb3}/Df(1)64c4$ flies are of normal viability (Table 1). However, such cytological observations could be inaccurate at this level of resolution.

Do any known alleles correspond to the null state of *zeste*? The results reported here are in accordance with the hypothesis that z^a mutations represent the inactive state of *zeste*. The eye color of *zeste*-deleted females is similar to that of $z^a/Df(1)w^{rJ1}$ females. Just as z^a adults, the morphology of *zeste*-deleted females appears normal. z^a mutations are viable and fertile in hemizygous males and homozygous females; we have shown that the *zeste* gene is not essential to viability. Finally, recent observations from our laboratory in-

TABLE 4

Addition of $P[z^+]$ to *zeste*-deleted females does not improve viability

Progeny class	Number	Percent
$Df(1)z^{deb3}/Df(1)w^{rJ1}$; $P[tko^+]_{22.1}/2$; $P[z^+]_{B1}/3$ ♀[a]	7	0.6
$Df(1)z^{deb3}/Df(1)w^{rJ1}$; $P[tko^+]_{22.1}/2$; 3/3 ♀[b]	18	1.4
$FM7a/Df(1)w^{rJ1}$; $P[tko^+]_{22.1}/2$ ♀[c]	466	36.7
$Df(1)z^{deb3}/y^+w^+B^s\cdot Y$; $P[tko^+]_{22.1}/2$ ♂[c]	372	29.2
$Df(1)w^{rJ1}/y^+w^+B^s\cdot Y$; $P[tko^+]_{22.1}/2$ ♂[c]	315	24.8
$Df(1)z^{deb3}/Df(1)w^{rJ1}/y^+w^+B^s\cdot Y$; $P[tko^+]_{22.1}/2$ ♀[c,d]	93	7.3
Total	1271	

Results of a cross between $Df(1)z^{deb3}/FM7a$; $P[tko^+]_{22.1}/P[tko^+]_{22.1}$; 3/3 females and an equal mixture of $Df(1)w^{rJ1}/y^+w^+B^s\cdot Y$; 2/2; $P[z^+]_{B1}/3$ and phenotypically indistinguishable $Df(1)w^{rJ1}/y^+w^+B^s\cdot Y$; 2/2; 3/3 males is tabulated here. The same cross was repeated with individual males, yielding similar results, as discussed in MATERIALS AND METHODS.

[a] Bright red eye pigmentation.

[b] Dull red eye pigmentation.

[c] Presence or absence of $P[z^+]_{B1}$ cannot be discriminated.

[d] Product of maternal nondisjunction, presumably due to presence of unmarked *Y* chromosome (see Table 2).

dicate that a domain at the C terminus of the *zeste* product is required for stimulation of *Ubx* expression in Drosophila tissue culture cell cotransfection experiments (A. CRICKMORE and M. L. GOLDBERG, in preparation). This region is not synthesized as part of the *zeste* protein produced by the z^a-like allele *In(1)e(bx)* (PIRROTTA *et al.* 1987; MANSUKHANI *et al.* 1988a), implying that this mutation is associated with an inactive *zeste* locus.

In contrast with this view, recent observations made in our laboratory during the course of these experiments suggest that the *zeste* allele z^{v77h}, distinguishable by several genetic criteria from z^a, is an alternative candidate for a null mutation. Polymerase chain reaction sequencing shows that the lesion associated with z^{v77h} is a 314-bp deletion which removes the initiation codon as well as a large portion of the untranslated upstream leader in the *zeste* mRNA (A. GRIMBERG and M. L. GOLDBERG, unpublished data). Previous studies of this mutant were of insufficient resolution to determine whether the initiation codon was in fact deleted (PIRROTTA *et al.* 1987; MANSUKHANI *et al.* 1988a). The molecular characterization of z^{v77h} remains incomplete: the sequence data to date do not rule out the possibility of additional alterations, and it is not clear if this allele produces any type of *zeste*-related polypeptide. Current information nonetheless indicates that if a protein were encoded by z^{v77h}, it should initiate from an AUG codon internal to the normal coding sequence, resulting in a potentially nonfunctional molecule lacking the N-terminal DNA-binding domain. Interestingly, the eye color phenotypes of z^{v77h} hemizygous males or homozygous females are similar to the dull reddish-brown eye color of *zeste*-deleted females, though it must be remembered that these "zesteless" flies were constructed with only a single dose of w^+. The results reported here do not establish whether z^a or z^{v77h} represents the null state; in fact, complete absence of *zeste* activity might have quantitative effects on Drosophila viability not shared by either allele.

Function of the *zeste* gene product: The results reported here show that the product of the *zeste* locus is not essential to Drosophila viability or to female fertility. This lack of a requirement for *zeste* is demonstrated even when the *zeste* product is provided neither as a maternal contribution in the egg nor as a product of the zygotic nucleus. Although we do not understand the relative infrequency at which *zeste*-deleted flies eclose, the survivors clearly cannot be ascribed as escapers from a "leaky" mutation, given the absence of *zeste*-coding sequences in these flies. On the other hand, because of the lowered rate of survival, the possibility that *zeste* expression may have quantitative effects on robustness cannot be ignored.

The conclusion that *zeste* is nonessential is surprising in light of evidence that it may participate in the regulation of a wide variety of target genes. Genetic interactions of *zeste* with at least three unlinked loci of apparently unrelated functions (*w*, *dpp*, and *Ubx*) have been well documented (LEWIS 1954; JACK and JUDD 1979; GELBART 1982; GELBART and WU 1982). These genetic effects appear in at least one case to reflect alterations in the transcription of target genes (BINGHAM and ZACHAR 1985), presumably through *zeste* protein activity as a DNA binding protein and as a transcription factor (BENSON and PIRROTTA 1987; MANSUKHANI *et al.* 1988b; BIGGIN *et al.* 1988). Over 60 *zeste* binding sites on salivary gland polytene chromosomes have recently been reported (PIRROTTA, BICKEL and MARIANI 1988). If a substantial fraction of these sites actually corresponded to loci regulated by *zeste*, the absence of the *zeste* locus might be expected to lead to more severe defects than those observed.

We suggest that these findings are best explained if, at most loci influenced by *zeste*, *zeste* protein is either a weak transcription factor, or that its function can be substituted by that of other regulatory proteins. Such an interpretation is also indicated by the behavior of the neomorphic alleles z^1 and z^{OP6}. Extending previous observations of BINGHAM and ZACHAR (1985), we have determined that there is a 5–8-fold reduction in steady-state levels of *white* mRNAs in the heads of flies exhibiting the phenotypes determined by these mutations (GUNARATNE 1987). Again, if the altered *zeste* protein produced by these alleles misregulated many additional target genes to the same extent, it is difficult to explain the lack of other

morphological defects or of obvious effects on viability.

zeste-deleted females are Ubx$^+$ in phenotype, despite recent reports that *zeste* protein enhances *in vitro* transcription of the *Ubx* gene (BIGGIN *et al.* 1988). Even moderate alterations in *Ubx* expression should have been visible, as reduction of *Ubx* gene dosage from two to one displays a dominant partial transformation of haltere toward wing. To explain this discrepancy, we propose either that *zeste* normally influences *Ubx* expression only very weakly if at all, at least during haltere development. Alternatively, the lack of *zeste* activity can be compensated by other gene products, perhaps reflecting some type of autoregulation at *Ubx*. The transvection-sensitive interactions between *zeste* and certain combinations of mutant alleles of the BX-C (LEWIS 1954) may reflect an abnormally sensitized state. In any event, it is clear that biochemical tests of transcription using extracts or purified components must be interpreted with caution. Though such experiments do suggest whether particular molecules might serve as transcription factors, additional genetic evidence is required to establish the physiological importance of the implied regulation.

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