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Mosaic suppressor, a Gene in Drosophila That Modifies Retrotransposon Expression and Interacts With *zeste*

Amy K. Csink,* Richard Linsk[†] and James A. Birchler*,[†]

**Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211 and* [†]*The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138*

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ABSTRACT

A newly identified locus in *Drosophila melanogaster*, *Mosaic suppressor* (*Msu*), is described. This gene modifies the expression of *white-apricot* (w^a), which is a *copia* retrotransposon-induced allele of the *white* gene. In addition to suppressing w^a in a mosaic fashion, this mutation suppresses or enhances the expression of several other retrotransposon induced *white* alleles. Mutations in *Msu* alter copia transcript abundance and may regulate the expression of several other retrotransposons. While each of the two *Msu* isolates is homozygous lethal, heteroallelic escapers occur at a low frequency. These escapers act not only as strong suppressors of w^a, but also as a recessive enhancer of synaptic-dependent gene expression at *white*. The mutation described here suggests a connection between the regulation of specific transcriptional units such as retrotransposons and more global synapsis dependent regulatory effects.

OUR laboratory is interested in dosage-sensitive modifiers of gene expression and their involvement in various phenomena such as suppression and enhancement, dosage compensation and aneuploid syndromes. In particular, we concentrate on a collection of regulatory loci that are implicated in regulating the expression of the *white* (*w*) eye color locus and the retrotransposon, *copia* in *Drosophila melanogaster*. The impetus for these studies derives from the observation that aneuploid segments produce a trans-acting dosage effect on unlinked genes (BIRCHLER 1979; BIRCHLER *et al.* 1990; RABINOW *et al.* 1991). To test whether such effects could be reduced to the action of single genes, mutageneses were conducted to test for dominant modifiers of the *white-apricot* (w^a) allele of the *white* eye color locus. *White-apricot* is caused by the parallel insertion of the retrotransposon *copia* into the second intron of *white* (GEHRING and PARO 1980; BINGHAM and JUDD 1981). Modifiers that alter the expression of *white* or copia, or affect the splicing or termination of the *copia* element within *white*, will potentially alter the phenotype of w^a.

A fraction of these modifiers has been described elsewhere (BIRCHLER and HIEBERT 1989; BIRCHLER *et al.* 1989; RABINOW and BIRCHLER 1989; RABINOW *et al.* 1991). In this paper we describe an unusual modifier, *Mosaic suppressor* (*Msu*), which suppresses *white-apricot* in a mosaic fashion with the suppression being more intense in the posterior portion of the eye. *Msu* alters copia transcript abundance and, since it also modifies several other retrotransposon-induced *white* mutants, may regulate the expression of the retrotransposons that cause those mutations as well. In addition, *Msu* modifies the phenotype of the regulatory gene *zeste*, which encodes a DNA binding protein that alters the expression of several genes including *bithorax* (KAUFMAN *et al.* 1973), *white* (GANS 1953) and *decapentaplegic* (GELBART 1982; GELBART and WU 1982) in a synapsis dependent fashion. The mutation described here demonstrates a connection between the regulation of retroviral-like elements and synapsis dependent regulatory effects.

MATERIALS AND METHODS

Fly stocks and cytology: Fly stocks were maintained on cornmeal-glucose-yeast media at 25°. The mutageneses which produced the two *Msu* alleles have been previously described (BIRCHLER *et al.* 1989; RABINOW *et al.* 1991). Larval salivary glands were dissected in 40% acetic acid and the polytene chromosomes stained with aceto-orcein.

RNA extraction: RNA was extracted using a guanidine-HCL method (COX 1968). Approximately 0.5 g of 2–24 hr adult flies were homogenized in 6 ml 8 M guanidine-HCL and precipitated with 50% ethanol. The RNA pellet was resuspended in 4 M guanidine-HCL and precipitated with 50% ethanol four times. The RNA pellet was extracted three times with sterile water, the extractions pooled and precipitated with 0.3 M sodium acetate and 75% ethanol. The resulting pellet was dissolved in sterile water to the appropriate concentration.

Northern blots: Twenty μg of total cellular RNA were electrophoresed through 1.5% agarose formaldehyde gels (LEHRACH *et al.*, 1977) and transferred to Biotrans nylon filters (ICN Inc.) according to manufacturers' instructions. Filters were prehybridized for 8 hr at 60° in 50% formamide, 5× SSC, 10× Denhardts, 0.5% SDS, 0.2 mg/ml sonicated, single stranded salmon sperm DNA and 9% dextran sulfate (for reagents see (MANIATIS *et al.* 1982)). Radioactive RNA probes (see below) were then added to a final concentration of 1.5 million cpm/ml and the hybridization contin-

ued for 20 hr at 60°. Filters were washed in 0.2× SSC, 0.05% SDS, at 75° for 30 min three times and exposed to X-ray film for autoradiography. Quantitation of the bands on the Northern blot autoradiographs was carried out using a LKB Ultrascan XL densitometer. The exposures scanned are not necessarily those shown in Figure 6, since, in some cases, shorter exposure times were used to ensure that the linear range of the film was not exceeded.

Antisense RNA probes used in this study have been described previously. The partial *white* cDNA probe, pATe1, which spans exons 3–6, and the genomic *white* subclone pBS12.5Xh-Pv, which spans part of intron 1 and exon 2, were used to analyze the *white* transcripts (BIRCHLER *et al.* 1989). *Copia* RNA was probed using the *Apa*I-*Hind*III copia fragment cloned into pIBI (RABINOW and BIRCHLER 1989). M. GOLDBERG provided a plasmid containing the cDNA clone of *zeste* (MANSUKHANI *et al.* 1988). Plasmid pBSBT1 was used to probe the blots with *β1tubulin* (BIALOGAN *et al.* 1985). T7 or T3 RNA polymerase (Promega) was used to incorporate ^{32}P-UTP into antisense RNA according to manufacturers' instructions.

RESULTS

In a chemical mutagenesis involving ethyl methane sulfonate (EMS) (BIRCHLER *et al.* 1989), a female fly carrying w^a on the *X* chromosome was found that had a mottled stripe of brilliant red at the posterior of the eye on an otherwise apricot background. The mutation producing this phenotype proved to be heritable and was located to the third chromosome. Because of the nature of the mutation it was termed, *Mosaic suppressor* (*Msu*). Years later in the course of a gamma irradiation mutagenesis (RABINOW *et al.* 1991), a similar mutation (Msu^2) was recovered. Each of these chromosomes by themselves contains a recessive lethal but heteroallelic Msu/Msu^2 flies are recovered at a frequency of approximately 4% of the expected value. This heteroallelic genotype very strongly suppresses the apricot phenotype (Figure 1a). Males and females of the genotype Msu/Msu^2 are completely sterile when allowed to mate with the opposite sex from a wild-type Canton-S stock. Interestingly, male heteroallelic escapers are recovered at about 50% lower frequency than female escapers.

In addition to the suppression in the eye, testes were also examined for any effect since *white* is expressed greatly in that tissue as well. With both *Msu* and Msu^2, there is an increase in pigment of the testes of males over siblings, both of which are carrying w^a on their *X* chromosomes (data not shown).

Modification by *Msu* of specific *white* alleles: To gain some initial information about the nature of this suppression, the two different chromosomes were tested for an effect on forty different alleles of the *white* locus. This collection of alleles includes various types of transposable element insertions, point mutations, promoter lesions, rearrangement alleles and an *Adh* promoter-*white* reporter construct. The rationale for this screen is that modifiers that are effective on all the structural gene lesions are implicated in affecting the *white* locus while those specific for transposable element insertions are believed to affect the phenotype because of an interaction with the respective elements. These tests were performed by simply crossing females carrying the various *X*-linked *white* alleles to males that were *Msu* or Msu^2 over a marked balancer and comparing the eye color of the F_1 *Msu*/+ or Msu^2/+ males to their Bal/+ brothers. The results are shown in Table 1 and the major points are summarized below.

There is a mosaic suppression of three alleles that were tested: w^a, w^{a4} and *white-roo-in-copia*. w^a and w^{a4} are both retrotransposon insertions in the second intron of *white*; the former being a *copia* (BINGHAM and JUDD 1981) element and the latter, *BEL* (ZACHAR and BINGHAM 1982; GOLDBERG *et al.* 1983). w^{ric} is a secondary insertion of a *B104* retrotransposon into the *copia* in w^a. In addition, there was a more subtle and nonmosaic suppression of *white-buff*, *white-buff2* and *white-honey* (Figure 1b). *White-buff* is an insertion of a *B104* retrotransposon in the fourth intron (O'HARE *et al.* 1984). *White-honey* is a reversion allele of *white-one* (O'HARE *et al.* 1991) caused by a secondary insertion of a *B104* element into the *Doc* element of w^1 in the untranslated 5′ leader of *white* (DRIVER *et al.* 1989). *White-buff2* is caused by a *B104* element inserted eight nucleotides 5′ to the start of *white* translation (A. CSINK, unpublished data). Lastly, *Msu* enhances the phenotype of *white-spotted 55*, which is an insertion of the *mdg3* retrotransposon within the untranslated leader of *white* (ZACHAR and BINGHAM 1982; A. CSINK, unpublished data).

Also shown in Table 1 is the response of three different types of revertants of *white-apricot*. There is no effect on w^{aR59k1}, which is a solo LTR derivative of w^a (CARBONARE and GEHRING 1985). The level of pigment is quite high in this revertant so the test was extended to w^{aR59k1}/w^{1118} females. Since w^{1118} is a *white* deletion, the eye pigment is further reduced and it is easier to test for suppression. Even in this configuration, however, there is no elevation of the pigment level to that characteristic of homozygous females, indicating the lack of interaction between *Msu* and the $w^{aR59klt1}$ *copia* LTR. This result suggests that the sequences required for an effect of *Msu* are present in the body of the *copia* element rather than in the LTR. A second revertant is w^{aR84h}, which is an insertion allele in the 3′ LTR of *copia*. This allele is suppressed in the w^{aR84h}/w^{1118} configuration. The last revertant tested was w^{aRM}, an insertion allele in the 5′ LTR of *copia* (MOUNT *et al.* 1988). Interestingly, this w^a revertant is enhanced by *Msu* alleles.

To examine the allele specificity further, each of the responsive alleles was tested with the mutant combination Msu/Msu^2 by first producing a stock of the *white* allele with the respective modifier allele balanced

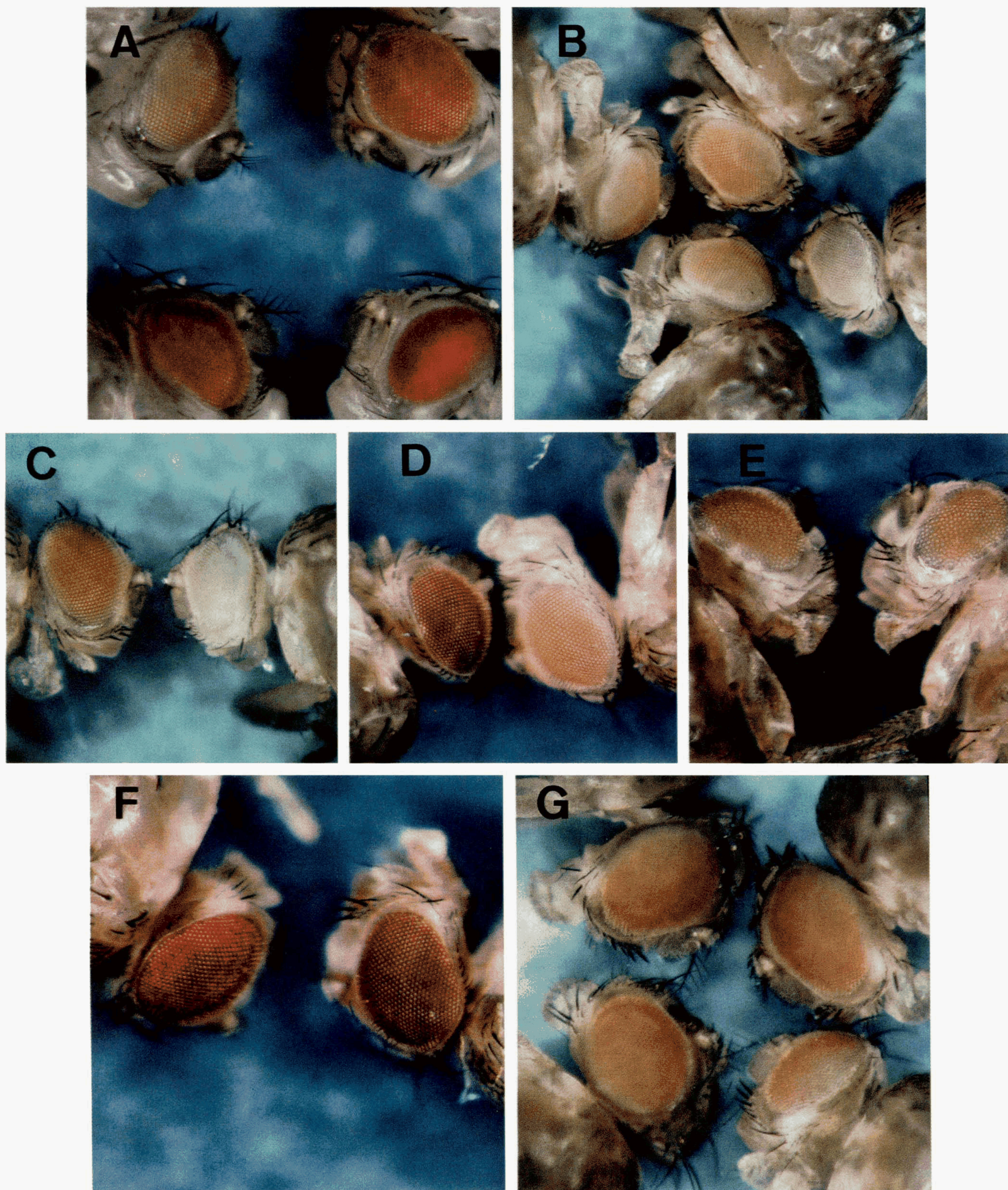

FIGURE 1.—Flies of the following genotypes (from top to bottom, left to right). (A) (top left) w^a; +/+, (top right) w^a; +/*Msu*, (bottom left) w^a; +/Msu^2, (bottom right) w^a; *Msu*/Msu^2. (B) (top left) w^{bf}; +/+, (top right) w^{bf}; +/*Msu*, (bottom left) w^{bf}; +/Msu^2, (bottom right) w^{bf}; *Msu*/Msu^2. (C) male (left) z^1 *Dp(1;1)*w^{+61e19}; +/+, (right) z^1 *Dp(1;1)*w^{+61e19}; *Msu*/Msu^2. (D) male (left) w^a; +/Msu^2, (right) w^a; +/+/Msu^2. (E) male (left) z^1 *Dp(1;1)*w^{+61e18}; +/Msu^2, (right) z^1 *Dp(1;1)*w^{+61e18}; +/Msu^2/*Msu*. (F) females (left) z^1 *Dp(1;1)*w^{+61e18}/+; +/Msu^2, (right) z^1 *Dp(1;1)*w^{+61e18}/+; Msu^2/*Msu*. (G) female (top left) w^{DZL}/+; +/+, (top right) w^{DZL}/+; +/*Msu*, (bottom left) w^{DZL}/+; +/Msu^2, (bottom right) w^{DZL}/+; *Msu*/Msu^2.

TABLE 1

Effect of *Msu* on selected alleles of the *white* gene

Allele	Interaction	*White* locus lesion	Reference
w^{a} (apricot)	+	*copia* retrotransposon insertion in second intron	Gehring and Paro (1980); Bingham and Judd (1981)
w^{a4} (apricot-4)	+	*BEL* retrotransposon in intron 2	Zachar and Bingham (1982); Goldberg, Paro and Gehring (1983)
w^{ric} (roo-in-copia)	+	Insertion of *B104* (*roo*) into *copia*	Davis, Shen and Judd (1987)
w^{bf} (buff)	* (non-mosaic)	*B104* in intron 4	Zachar and Bingham (1982); O'Hare *et al.* (1984)
w^{bf2} (buff-2)	* (non-mosaic)	*B104* in 5′ leader	A. Csink (unpublished data)
w^{h} (honey)	* (non-mosaic)	*B104* element into *Doc* element of w^{1}	O'Hare *et al.* (1991)
w^{sp55} (spotted-55)	−	*mdg3* retrotransposon in 5′ untranslated leader	Zachar and Bingham (1982); A. Csink (unpublished data)
w^{aRM} (apricot revertant)	−	Transposable element in *copia* 5′ LTR	Mount, Green and Rubin (1988)
w^{aR84h} (apricot revertant)	+ (non-mosaic)	*I* element insertion in *copia* 3′ LTR	Mount, Green and Rubin (1988)
w^{a59k1} (apricot revertant)	None	Solo *copia* LTR	Carbonare and Gehring (1985)
w^{i} (ivory)	None	Duplication of sequences from intron 1 to start of exon 3	Collins and Rubin (1982); Karess and Rubin (1982); O'Hare *et al.* (1984)
w^{c} (crimson)	None	*FB* insertion revertant of w^{1}	Collins and Rubin (1982); Karess and Rubin (1982); O'Hare *et al.* (1984)
w^{sp} (spotted)	None	*B104* retrotransposon in 5′ regulatory region	Zachar and Bingham (1982); O'Hare *et al.* (1983, 1984)
w^{sp4} (spotted-4)	None	Deficiency in 5′ regulatory region	Zachar and Bingham (1982); O'Hare *et al.* (1984)
w^{sp2} (spotted-2)	None	Deficiency in 5′ regulatory region	Zachar and Bingham (1982)
w^{sp81d5} (spotted-81d5)	None	Deficiency in 5′ regulatory region	Davison *et al.* (1985)
w^{co} (coral)	None	*coral* retrotransposon in intron 5	A. Csink (unpublished data)
w^{t} (tinged)	None	Unknown	
w^{ec3} (ecru-3)	None	Unknown	
w^{mo} (mottled-orange)	None	Unknown	
w^{cf} (coffee)	None	Point	Zachar and Bingham (1982)
w^{a2} (apricot-2)	None	Point	Zachar and Bingham (1982)
w^{a3} (apricot-3)	None	Point	Zachar and Bingham (1982)
w^{sat} (satuma)	None	Point	Zachar and Bingham (1982)
w^{col} (colored)	None	Point	Zachar and Bingham (1982)
w^{Bwx} (Brownex)	None	Point	Zachar and Bingham (1982)
w^{bl} (blood)	None	*blood* retrotransposon in intron 2	Zachar and Bingham (1982); Bingham and Chapman (1986)
w^{e} (eosin)	None	Transposable element reversion of w^{1} (*Doc* element)	Zachar and Bingham (1982); O'Hare *et al.* (1984); Hazelrigg (1987)
w^{apl} (apricot-like)	None	P-M hybrid dysgenic revertant of w^{1} (*Doc* element)	C. McElwain (unpublished data)
w^{IR1}	None	*I* element insertion	Sang *et al.* (1984)
w^{IR2}	None	*I* element insertion revertant of w^{1} (*Doc* element)	Sang *et al.* (1984)
w^{IR3}	None	*I* element insertion	Sang *et al.* (1984)
w^{IR4}	None	*I* element insertion	Sang *et al.* (1984)
w^{IR5}	None	*I* element insertion	Sang *et al.* (1984)
w^{IR6}	None	*I* element insertion	Sang *et al.* (1984)
$z\ w^{is}$ (isoxanthopterinless)	None	Unknown	
w^{zm} (zeste-mottled)	None	*BEL* retrotransposon in intron 1	Zachar and Bingham (1982); O'Hare *et al.* (1984)
$z\ w^{zl}$ (zeste-light)	None	Derivative of w^{zm}	Judd (1963)
$z\ w^{zm}$	None	*BEL* retrotransposon in intron 1	Zachar and Bingham (1982); O'Hare *et al.* (1984)
$z\ w^{a}$	None	*copia* insertion in intron 2	
z Dp(1;1)w^{+61e19}	None	Duplication of *white* locus sequences	Green (1963); Gunaratne *et al.* (1986)
Adh-w #2	None	*Adh* promoter-*white* structural gene on chromosome *3*	Birchler *et al.* (1990)

Except where noted, + denotes mosaic suppression in +/*Msu*, * denotes *Msu*/+ suppression and *Msu*/Msu^{2} enhancement, − denotes enhancement.

on the third chromosome. In addition to the responsive alleles, two others were included (*white-zeste-mottled* and *white-zeste-light*). They are caused by *BEL* insertion, as is w^{a4}, in the first large intron of *white* (O'HARE *et al.* 1984) but are phenotypically dark and might not have been recognized as being affected by the heterozygous *Msu* or Msu^2. The Msu/Msu^2 combination strongly suppresses *white-apricot, apricot4* and *ric,* while further enhancing w^{sp55}. In contrast, this combination strongly enhanced the phenotype of *white-buff, buff2* and *honey,* producing a bleach white color even though they were suppressed by the heterozygotes (Figure 1b).

Msu enhancement of the zeste effect: The effect of the *zeste* gene on *white* is an example of pairing dependent regulation. A recessive, X-linked, gain-of-function mutation in the *zeste* locus, *zeste-one,* gives w^+ females with bright yellow-orange eyes. However, the males carrying the z^1 allele have wild-type eyes. The mutant effect can be seen in males if they carry a tandem duplication of w^+. Therefore, two copies of *w* in close association are required for the action of z^1 (JACK and JUDD 1979).

As noted above, we tested two other alleles, *white-zeste-light* and *white-zeste-mottled.* Alone their phenotype is basically wild type, but in the presence of z^1 these *w* alleles produce a mottled phenotype. w^{zm} was tested without z^1 in the genotype while the chromosome with w^{zl} had z^1. Interestingly, while there is no observable effect in the heterozygotes of *Msu,* the heteroallelic combination produced white eyes with the z^1 w^{zl} chromosome but had no effect on w^{zm}. This result could be interpreted in at least two ways. Either the *Msu* mutation is a recessive enhancer of z^1, or a specific enhancer of the w^{zl} allele, perhaps with the requirement for z^1.

To test these possibilities, several *X* chromosomes were substituted into the balanced *Msu* and Msu^2 stocks. These included $zeste^1$ *Dp(1;1)* w^{+61e19} (GREEN 1963), $zeste^1$, and Canton-S wild type. The respective *X* chromosome stocks with opposite alleles of *Msu* were crossed together and the heteroallelic class scored for an effect on eye color. The *zeste-white* duplication stock is enhanced in both sexes. The *zeste* stock alone is enhanced in females and has no effect in males. The Canton S *X* chromosome is normal in eye color. The results of these experiments indicate that *Msu* interacts with the *zeste* effect in the above test as opposed to the retroelement insertion (Figure 1c).

The interaction with the *zeste* phenomenon was examined further. BINGHAM and ZACHAR (1985) characterized an allele of *white,* referred to as *Dominant zeste like* (w^{DZL}), which produces a mutant phenotype as a heterozygote in females and a much less mutant phenotype in hemizygous males. This allele was incorporated into a balanced stock of Msu^2. The new stock was then crossed by the Canton S, *Msu* stock. In heterozygous w^{DZL}/+ (Canton S) females, the Msu/Msu^2 genotype enhances w^{DZL}, while the other three genotypes (*Msu*/+, Msu^2/+ and +/+) appear identical (Figure 1g). The males that were Msu/Msu^2 were not enhanced.

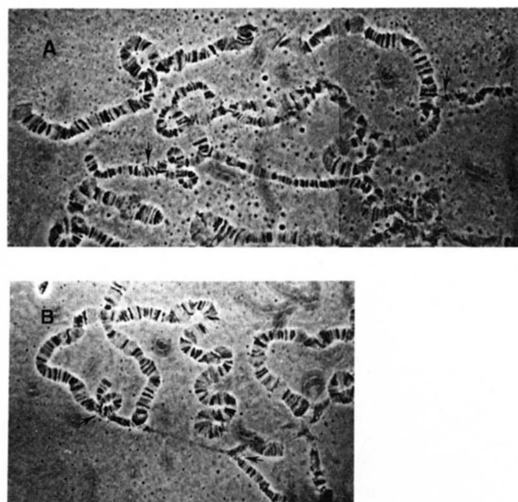

FIGURE 2.—Polytene larval salivary glands from (A) *Msu*/+ and (B) Msu^2/+. Arrows indicate the 98F breakpoint.

To test whether the *z* mutation could be made dominant by the enhancing effect of *Msu,* females were produced that were heterozygous for Canton S wild type and the *z* mutation in the presence of Msu/Msu^2. The *z*/+; Msu/Msu^2 did not appear to be any different than the other classes, indicating that the enhancement effect does not produce a dominant effect.

Localization of the *Msu* gene: Attempts to map the Msu^2 allele against two different multiply marked third chromosomes suggested that the chromosome carried a rearrangement in the right arm and that the mutation was associated with it. Use of the *Msu* chromosome in recombination experiments also indicated the presence of a rearranged chromosome. Cytological examination of polytene chromosomes from both *Msu* alleles revealed a number of breakpoints on the third chromosome (Figure 2). We found inversions with breakpoints near the centric heterochromatin and 98F in both alleles, although there were other breaks in the third chromosome unique to each allele.

To examine the nature of the *Msu* mutations at the 98F breakpoint, we performed two crosses using a Y-autosome translocation line (*T(Y;3)R128*) to produce a partial trisomic from 97F to the end of 3R (LINDSLEY *et al.* 1972). The first cross (Figure 3) produced a partial trisomic that contained *Msu* and two wild-type copies of the 98F region. This genotype no longer suppressed w^a, although, of course, the heterozygous euploid did (Figure 1d). The second cross (Figure 4) was designed to determine whether this region had

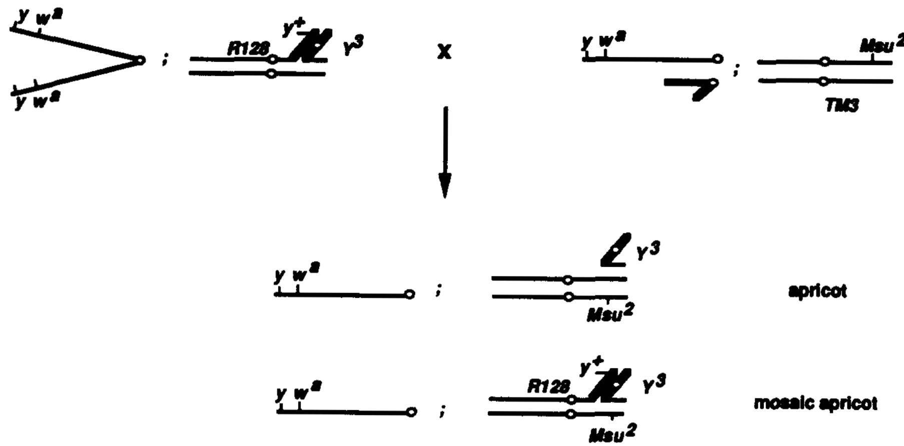

FIGURE 3.—Diagram of the cross (*C(1)RM, y w^a; T(Y;3) R128* × *y w^a; Msu^2/TM3, Ser*) to determine the effect on the *Msu* suppression of w^a of trisomy for the region around the 98F breakpoint. The thin lines represent chromosomes *3* and *X*. The thicker lines represent the *Y* chromosome. Circles represent centromeres.

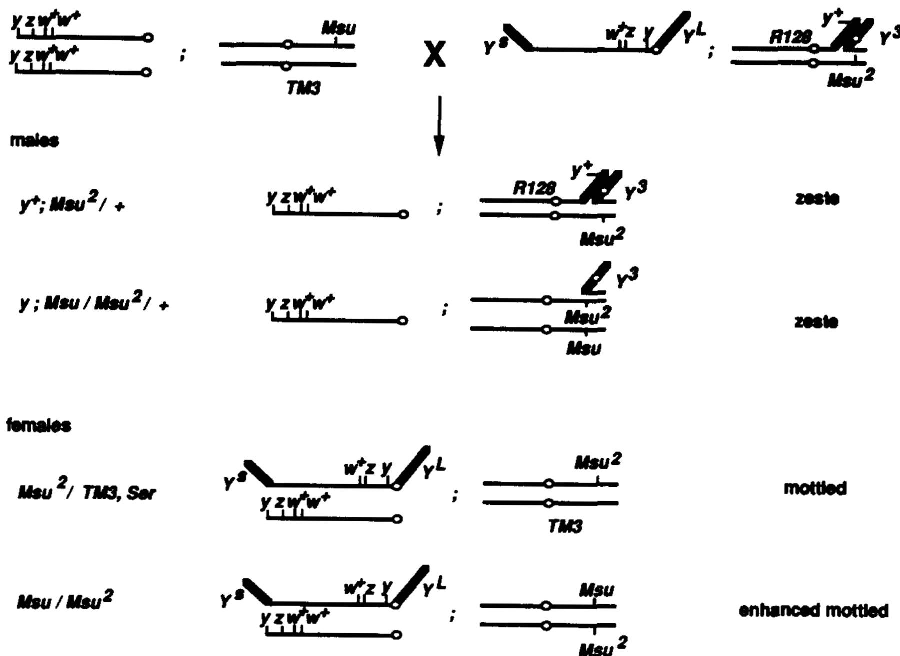

FIGURE 4.—Diagram of the cross (*y z^1 Dp(1;1) w+; Msu/TM3, Sb, Ser* × *$Y^SX \cdot Y^L$, In(1) EN, y; Msu^2/T(Y;3) R128 y^+* to determine the effect on the *Msu* recessive enhancement of *z* of trisomy for the region around the 98F breakpoint. The thin lines represent chromosomes *3* and *X*. The thicker lines represent the *Y* chromosome. Circles represent centromeres.

any effect on the recessive enhancement of *zeste*. *Msu/Msu^2/+* males from this cross do not enhance *zeste* (Figure 1e). The females from this same cross provide a useful control in the following way. JACK and JUDD (1979) showed that in females containing w^+/*z Dp(1;1)*w^+, *zeste* became slightly dominant and the females had mottled orange eyes. *Msu/+* females show slight mottling and the *Msu/Msu^2* females, a greater amount, demonstrating that *Msu/Msu^2* enhances the *zeste* effect under these circumstances also (Figure 1f). However, the females in our cross did not display the *zeste* phenotype as strongly as those in the earlier study probably because one of the *X* chromosomes in our cross was inverted, therefore inhibiting pairing among the three *white* genes. These results suggest that the suppression of w^a and enhancement of *z* are due to loss-of-function alleles at *Msu* and are consistent with the common 98F breakpoint in the two alleles being the responsible lesion for both mutational effects.

We also localized the *Msu* gene by deficiency mapping using *Df(3R)3450* (98E3;99A6-8). Females that were w^a; *Msu^2/TM3, Ser* were crossed to w^{1118}; *Df(3R)3450/MKRS* males and the w^a/*Y*; *Msu^2/Df(3R)3450* progeny were found to suppress w^a to almost wild type. Additionally, we crossed *z Dp(1;1)*w^{+61e19}; *Msu^2/TM3, Sb Ser* females to the above deficiency males and obtained white eyed flies of the genotype *z Dp(1;1)*w^{+61e19}/*Y*; *Msu^2/Df(3R)3450*. This result shows that *Msu^2/Df(3R)3450* enhances *z* in the same manner as the *Msu^2/Msu* heteroallelic escapers.

Because the breakpoint involved with *Msu* is extremely close if not coincident with the *Darkener of apricot* locus (RABINOW and BIRCHLER 1989), experiments were performed to test whether *Msu* is an allele of *Doa*. The first distinction between the two mutations is in the allele specificity at *white*. *Doa* suppresses w^a and enhances w^{sp55} and has no effect on any other *white* allele. As noted above, *Msu* effects a broader range of alleles. Second, when *Msu* and Msu^2 are crossed to the *Doa* alleles, *hd1* and *dem*, the degree of

suppression of is not to the level of wild type as is characteristic of *Msu* and *Doa* homozygous escapers (RABINOW and BIRCHLER 1989). The two combinations of mutations were also fully complementary with regard to viability in contrast to Msu/Msu^2 or Doa^{hd1}/Doa^{dem}. To test for complementation of the enhancement of *zeste*, the *Msu* and Msu^2 stocks carrying the *X* chromosome with *zeste* and the *white* duplication, were crossed by the two *Doa* alleles and the male progeny scored for the enhancement of *zeste* in the *Msu/Doa* or Msu^2/Doa classes. None of the heterozygous combinations exhibited a *zeste* enhancement. Therefore, by these genetic criteria, *Msu* and *Doa* are discrete complementation groups, although these tests do not rule out a molecular relationship.

Attempted isolation of *Msu* revertants: Given the mosaic nature of the phenotype of *Msu*, attempts to revert the mutations were performed to test whether the original alleles were gain-of-function. The first attempt was by gamma irradiation, which generated several isolates that were reduced in pigment but only one which returned *Msu* to the normal apricot phenotype and none that reverted Msu^2. Subsequently, chemical mutagenesis was conducted with ethyl methane sulfonate (EMS) and a potential revertant of Msu^2 was recovered. Further analysis of these presumptive revertants revealed that a second site suppressor (not an *Msu* revertant) had been induced in both cases, that was present in the left arm of chromosome *3* and that could be recombined away. Since the *Msu* breakpoints for both the alleles were near the centric heterochromatin, we tested these second site mutations for suppression of position effect variegation (PEV) by combining them with *white-mottled-4h*. This *white* allele is caused by a rearrangement of the *X* chromosome that moves a wild-type *white* gene near heterochromatin and leads to a patchy *white* inactivation. We found that these second site suppressors of *Msu* were indeed suppressors of PEV. Thus, the basis of this suppression appears to be due to the reversal of position effect at the *Msu* locus and *Msu* may be subject to modification by other modifiers of PEV.

Effects of *Msu* on transcript levels of *white*, *zeste* and *copia*: We investigated the molecular basis of the action of *Msu* by examining the transcripts emanating from w^a, which is caused by a parallel insertion of the retrotransposon, *copia*, into the second intron of *white* (LEVIS *et al.* 1982). The mutant effect is believed to occur when *white* initiated transcripts terminate in the second LTR of *copia*. However, this termination is not absolute, resulting in a low level of transcripts that continue to the 3′ terminus of *white*. The *copia* element is spliced out along with the remainder of intron 2, resulting in a low level of functional normal sized messenger RNA. A diagram of *white-apricot* is shown in Figure 5, with the stable transcripts (described in

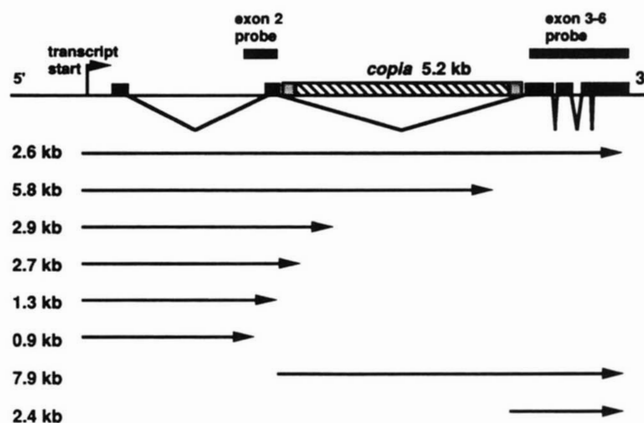

FIGURE 5.—Illustration of the w^a allele of the *white* locus and the transcripts it produces. The two probes used to determine the 5′ and 3′ originating transcripts are shown.

ZACHAR *et al.* 1985; MOUNT *et al.* 1988; HIEBERT and BIRCHLER 1992) and probes used in this study noted.

To analyze the effect of *Msu* on w^a, a segregating population was generated by crossing flies, homozygous on the *X* chromosome for w^a, but heterozygous for *Msu*/+ in one parent and Msu^2/+ in the other. The progeny were classified as Msu/Msu^2, *Msu* or Msu^2/+ and +/+. RNA was isolated from each progeny class and used in Northern blots that were probed with *white* gene probes 5′ and 3′ to the *copia* element in *white*, followed by a loading control using *β1-tubulin*. The Northern blots are shown in Figure 6, a and b. The probe 3′ (exon 4–6, Figure 5) to *copia* can detect three transcripts: (1) the normal sized *white* message (2.6 kb); (2) a transcript, present at low levels, initiated in the 5′ LTR of *copia*, reading through the 3′ LTR and terminating in *white* (7.9 kb) and (3) a transcript, rare in adults (not seen in these Northern blots), initiated in the 3′ LTR of the *copia* element and terminating in *white* (2.4 kb). The two transcripts detected here are in greatest abundance in Msu/Msu^2 flies, intermediate in heterozygotes and lowest in +/+ (Figure 6a, Table 2). This is consistent with the phenotypic effect of *Msu* on w^a, in that the pigment levels are correlated with the level of the 2.6-kb transcript. This result suggests that *Msu* produces a dosage effect on the RNA level of these *white* transcripts that is inversely correlated with the number of functional *Msu* alleles present.

The 5′ probe used involves the second exon of *white* (Figure 5). The RNAs detected with this probe are those initiated at *white* and terminated in the 5′ LTR (0.9 kb) and 3′ LTR (5.8 kb). Three other transcripts are spliced or terminated within *copia* (1.3 kb, 2.7 kb and 2.9 kb) and a low level of normal sized *white* message (2.6 kb) is also detected. The most abundant of these RNA's is the 3′ LTR terminated species, which is reduced slightly in the Msu/Msu^2 males, but which is otherwise unaffected (Figure 6b, Table 2). These observations suggest that *Msu* has little or no

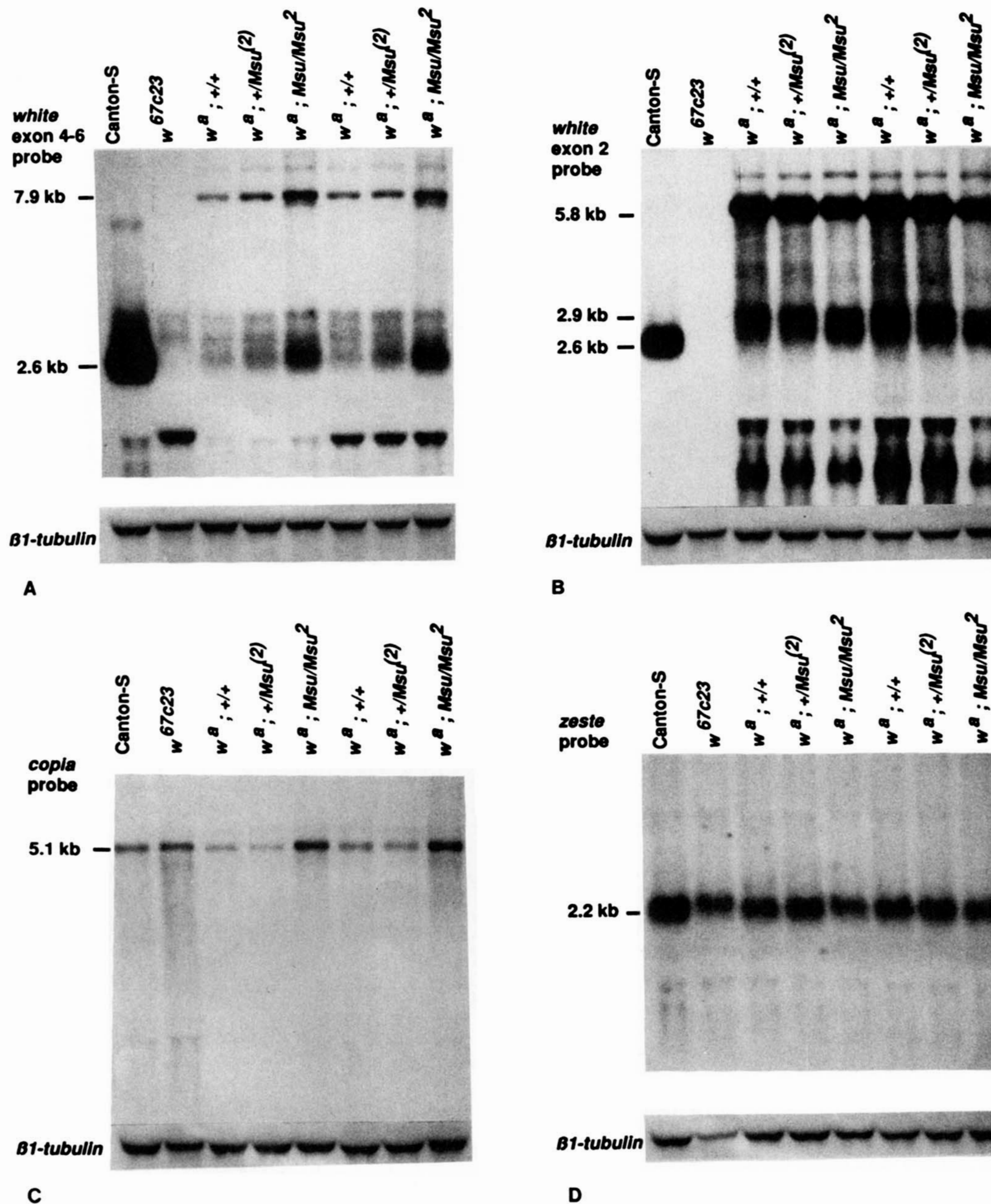

FIGURE 6.—Northern blots of total cellular RNA probed with (A) 3′ *white* (exon 4–6), (B) 5′ *white* (exon 2), (C) *copia*, (D) *zeste* (see MATERIALS AND METHODS for description of probes). The bottom panel of each blot shows the reprobing with *β1-tubulin* as a loading control. Genotypes are indicated at the top of the lane. The lanes marked $Msu^{(2)}/+$ contain a mixture of $Msu/+$ and $Msu^2/+$. The Canton-S flies were female, the w^{67c23} (a *white* deletion mutant) were males, the first three lanes from the *Msu* segregating population were females and the last three male. In blot B using the 5′ *white* probe the 2.6-kb *white* transcript is not visible in the *Msu* segregating population because of the short exposure time and the fact that it is obscured by the 2.7-kb band. The transcripts common to the deficiency w^{67c23} and the other Northern lanes are not due to transcription from the *white* locus.

modulating effect on the *white* promoter, but is acting via the *copia* element in the second intron.

To examine further whether there is evidence for the modulation of *copia*, total *copia* RNA was estimated in the above genotypes. Indeed, total *copia* RNA is elevated 2–3 fold in the Msu/Msu^2 class relative to +/+. However, the heterozygous class shows a slight reduction (20–35%) relative to the wild-type individuals (Figure 6c, Tables 2 and 3). This is slightly different than the predicted effect based on the response of the *copia* at *white*, but has been observed in four replicate gels (Table 3). The basis of this discrepancy between the *copia* at *white* and the total population is not known. We do note, however, that there is also a nonadditive heterozygous effect on the phenotypic level with the *white* alleles *buff*, *buff2* and *honey* albeit in the opposite direction.

It should be pointed out that these measurements were performed on segregating populations. Therefore, any differences in retrotransposon transcription

TABLE 2

Densitometric quantitation of northerns (Figure 6) of RNA from segregating *Msu* genotypes

Genotype	Sex	*copia*	*white* wild type (2.6 kb)	*white* 5′ w^a (5.8 kb)	*white* 3′ w^a (7.9 kb)	*zeste*
Canton-S	Female	0.532	1.000			1.000
w^{67c23}	Male	0.934				0.598
+/+	Female	0.300	0.017	0.573	0.272	0.570
Msu or Msu^2/+	Female	0.230	0.030	0.596	0.503	0.723
Msu/Msu^2	Female	1.000	0.099	0.579	1.000	0.530
+/+	Male	0.512	0.028	1.000	0.465	0.705
Msu or Msu^2/+	Male	0.395	0.059	0.899	0.463	0.674
Msu/Msu^2	Male	0.905	0.169	0.481	0.885	0.421

The densities of the bands on the autoradiographs of the Northern blots were determined using an LKB Ultrascan XL densitometer and the area under the peaks determined using the Pharmacia GelScanXL analysis program. All values were corrected for loading variation by reprobing each blot with a β_1-*tubulin* probe and dividing each value by the intensity of the β_1-*tubulin* hybridization in that lane. The units are arbitrary, with the highest value for each probe being 1.000. The probe covering *white* exons 3–6 (see Figure 5) was used to detect the messages 3′ of the *copia* insert as well as the wild type *white* message (Figure 6a) and the probe covering *white* exon 2 used to detect the messages 5′ of the *copia* insert (Figure 6b).

TABLE 3

Densitometric quantitation of Northern blots to determine the effect of *Msu* on *copia* RNA abundance (n = 4)

Genotype	Sex	*Copia* (Mean ± S.D.)
Canton-S	Female	1.45 ± 0.26
w^{67c23}	Male	2.45 ± 0.25
+/+	Female	0.79 ± 0.09
Msu or Msu^2/+	Female	0.63 ± 0.13
Msu/Msu^2	Female	2.28 ± 0.11
+/+	Male	1.09 ± 0.03
Msu or Msu^2/+	Male	0.71 ± 0.04
Msu/Msu^2	Male	1.94 ± 0.12

The mean densitometric value for the 5.1-kb *copia* transcript from four replicate northern gels is shown with the standard deviation. Units are arbitrary. *Copia* values were determined relative to β_1-*tubulin* as described in Table 2.

due to variation in the original strains would be minimized by either independent assortment or, in the case of the third chromosome, recombination. Indeed, the original line used in the mutagenesis that produced Msu^2 was the w^a stock, which provided the wild-type chromosome in the segregating population. On the other hand, the *Msu* chromosome was derived from a different line and contains 3R inversions, so recombination cannot homogenize this chromosome arm. However, we do not think this fact compromises the results for the following reasons. If the differences between *copia* transcription in the +/+ and Msu/Msu^2 flies were due to variation in the activity of copia elements on 3R (*i.e.*, the *Msu* chromosome contained especially active elements) then the expected *copia* transcript abundance in the *Msu*/+ should be intermediate between the two homozygous classes. It is also possible that the parental chromosome on which *Msu* was induced contained a *trans*-acting modifier of *copia*. However, such a hypothetical gene would not be homozygous in the heteroallelic combination (Msu/Msu^2). Half of the flies in the *Msu* or Msu^2/+ class would be of the same chromosome composition as the heteroallelic combination. Therefore, once again one would expect the heterozygous flies to be intermediate between the homozygotes. This is not the case. Secondarily induced or naturally occurring modifiers would not be homozygous in the Msu/Msu^2 combination. Therefore, we believe that the observed differences are due to the *Msu* mutation, acting directly or indirectly.

Since *Msu* is a recessive enhancer of *zeste* we tested for an effect of *Msu* on *zeste* RNA abundance. We found a slight (25–35%) decrease of *zeste* RNA in Msu/Msu^2 flies relative to $Msu^{(2)}$/+, but no major or significant change (Figure 6d, Table 2). Additionally, we tested for an effect of *Msu* on *zeste* transcript abundance in 1–2 day pupae, but again found no difference between *Msu*/+ and Msu/Msu^2 genotypes (data not shown).

DISCUSSION

Modifier genes can act upon their target loci in a great variety of ways. We have shown here that *Msu* interacts with the *copia* retrotransposon present in w^a. In addition, it interacts only with *white* alleles containing the *copia*, *BEL*, *B104* and *mdg3* retrotransposons, although some of these alleles contain additional insertions (the *I* and *Doc* retroposons). Other retrotransposon insertion alleles such as w^{bl} and w^{co} were tested, but were unaffected by *Msu*. Therefore, *Msu* is not a general modifier of retrotransposon insertions. Because the insertion sites of these elements are in different locations within the gene (except that none are found in the protein coding region, but all interrupt the primary transcript), it is unlikely that *Msu* is specific for the affected alleles due to their insertion site. Instead, *Msu* may interact with these five retrotransposons through a common regulatory mechanism.

Msu acts upon w^a in a dosage dependent manner, as has been shown for other modifiers of w^a, with the amount of suppression decreasing with the number of functional *Msu* alleles. However, it does not act on the total abundance of *copia* RNA in the same manner. The *Msu*/+ flies actually have slightly less copia transcripts than +/+ flies whereas those transcripts from the Msu/Msu^2 flies are greatly increased. Indeed, transcripts originating in the *copia* within the w^a allele seem to be behaving differently than the total pool of *copia* RNA in adults. We observe this in the 5′ LTR initiated *copia-white* fusion message from w^a (Figures

5 and 6a) which is affected in a dosage dependent manner. This discrepancy could indicate that there is an interaction among the w^a *copia*, *Msu* and the surrounding *white* gene. Interestingly, the effect of *Msu* on the *white* alleles *buff*, *buff-2* and *honey* follows the pattern of *copia* levels, in that *Msu*/+ has the directionally opposite effect of Msu/Msu^2, *i.e.*, *Msu*/+ suppresses these alleles, but Msu/Msu^2 enhances them both relative to +/+.

It is intriguing that *Msu*, a specific regulator of retrotransposon expression, is also involved in chromosomal pairing "dependent gene expression", as shown by its recessive enhancement of *zeste*. It has been shown that the *zeste* protein can serve as a transcriptional activator of the *Ubx* gene *in vitro* (BIGGIN *et al.* 1988), but its role *in vivo* is uncertain since flies deleted for the entire *zeste* gene are Ubx^+ (GOLDBERG *et al.* 1989). It is difficult to determine whether the small decrease in *zeste* RNA abundance in adult Msu/Msu^2 flies is relevant to the enhancement of *zeste*. While the decrease is consistent in males and females (as is the enhancement of *zeste*), it is not of the magnitude one would expect given the extreme effect of *Msu* on the *zeste* phenotype. Nor do we see a change in *zeste* RNA in pupae. However, it is quite possible that *zeste* RNA is more strongly effected by *Msu* in a subset of the tissues of the fly relevant to eye color, but does not dramatically alter the *zeste* RNA abundance in the majority of other tissues. In any case, it is uncertain how a change in the amount of product from the z^1 mutation, a recessive gain-of-function gene, would effect the phenotype. The observation that z^-/z^1 flies are *zeste* (unenhanced) (GANS 1953; JACK and JUDD 1979) suggests that a decrease of zeste RNA by half would have no effect on the *zeste* phenotype. Indeed, deletion of the entire *zeste* locus has little effect on eye color (GOLDBERG *et al.* 1989). The mechanism of enhancement of the *zeste* phenotype will require further study.

The fact that *Msu* modifies w^{DZL} without the presence of the z^1 mutation indicates that it is not merely interacting with z^1, but rather may play its own role in transvection. It is possible that the role of *Msu* in transvection involves interaction with the z^+ product. However, it is not simply an equivalent of $zeste^1$, since it has no effect on wild type *white* as does z^1. A number of other modifiers of *zeste* have been previously described including *Enhancer of zeste*, *Suppressor of zeste 2* and *Sex comb on midleg* (KALISCH and RASMUSON 1974; PERSSON 1976; WU *et al.* 1989; JONES and GELBART 1990). Interestingly, these loci produce other phenotypic effects that include them in the Polycomb-group of homeotic genes. However, all of these modifiers of *zeste* exhibit a dominant effect and *Msu* is therefore the first locus found that is a recessive enhancer of *zeste*.

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