The Effect of Modifiers of Position-Effect Variegation on the Variegation of Heterochromatic Genes of *Drosophila melanogaster*

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ABSTRACT

Dominant modifiers of position-effect variegation of *Drosophila melanogaster* were tested for their effects on the variegation of genes normally located in heterochromatin. These modifiers were previously isolated as strong suppressors of the variegation of euchromatic genes and have been postulated to encode structural components of heterochromatin or other products that influence chromosome condensation. While eight of the modifiers had weak or no detectable effects, six acted as enhancers of *light* (*lt*) variegation. The two modifiers with the strongest effects on *lt* were shown to also enhance the variegation of neighboring heterochromatic genes. These results suggest that the wild-type gene products of some modifiers of position-effect variegation are required for proper expression of genes normally located within or near the heterochromatin of chromosome 2. We conclude that these heterochromatic genes have fundamentally different regulatory requirements compared to those typical of euchromatic genes.

THE chromosomes of higher eukaryotes are comprised of both euchromatin and heterochromatin which can be distinguished cytologically by differences in levels of condensation. Regions known as "constitutive heterochromatin" appear highly condensed throughout the cell cycle (HEITZ 1928). Although heterochromatin comprises a large portion of the genome of some eukaryotes (reviewed in HILLIKER, APPELS and SCHALET 1980; JOHN 1988), it contains few known genetic functions compared to euchromatin. For those functions that have been identified, little is known of their regulatory requirements. However, one well documented phenomenon, position-effect variegation (PEV) has provided insight into the differences between heterochromatic and euchromatic genes. This phenomenon has been most extensively studied using Drosophila (reviewed in SPOFFORD 1976). Its generality for a large number of Drosophila genes is thought to reflect underlying structural and functional differences between euchromatin and heterochromatin.

PEV is observed as the mosaic expression of a gene that has been moved to a new location on the chromosome. Most examples involve variegation of a euchromatic gene displaced next to heterochromatin by chromosome rearrangement (SPOFFORD 1976; EISSENBERG 1989). Additional examples have been observed as transformed genes inserted next to heterochromatin (DANIELS *et al.* 1986; R. LEVIS, personal communication). It is most commonly believed that gene inactivation occurs at the transcriptional level (HENIKOFF 1981; RUSHLOW, BENDER and CHOVNICK 1984; KORNHER and KAUFFMAN 1986; HENIKOFF and DREESEN 1989) and is due to changes in chromosome structure created by the juxtaposition of euchromatic and heterochromatic sequences at the breakpoint. Mosaicism is thought to result from variation among cells in the distance that the condensed heterochromatic conformation propagates into euchromatin. Results from a number of other studies support the notion that changes in chromatin structure play a critical role in PEV. Cytological evidence has been obtained for changes in chromosome morphology induced by breakpoints causing variegation (CASPERSSON and SCHULTZ 1938; HARTMANN-GOLDSTEIN 1967; REUTER, WERNER and HOFFMANN 1982; HAYASHI *et al.* 1990). PEV can be modified by changes in histone gene dosage (KHESIN and LEIBOVITCH 1978; MOORE *et al.* 1979) or by drugs that are believed to affect histone modification (MOTTUS, REEVES and GRIGLIATTI 1980). Changes in the heterochromatic content of a nucleus, by the addition of the entirely heterochromatic *Y* chromosome for example, can also affect the degree of variegation (GOWAN and GAY 1933). The *Y* chromosome is thought to compete for heterochromatic proteins that would otherwise bind at the variegating breakpoint (ZUCKERKANDL 1974; REUTER, DORN and HOFFMAN 1982).

The proposed mechanism of PEV can be best tested by identifying the molecular components involved. One particularly promising approach has been to identify mutations that act in *trans* to suppress or enhance PEV. SCHULTZ was the first to screen for dominant modifiers of PEV (MORGAN, SCHULTZ and CURRY

1941). A detailed analysis of a single locus modifier of PEV was carried out by SPOFFORD (1967). Recently, more than 150 X-ray, *P* element or ethyl methanesulfonate (EMS)-induced dominant modifiers (*Su(var)*s) of position effect (HENIKOFF 1979; REUTER and WOLFF 1981; REUTER and SZIDONYA 1983; SINCLAIR, MOTTUS and GRIGLIATTI 1983; REUTER *et al.* 1986; REUTER *et al.* 1987; LOCKE, KOTARSKI and TARTOF 1988) have been characterized. It has been proposed that between 20 (LOCKE, KOTARSKI and TARTOF 1988) and 150 (WUSTMANN *et al.* 1989) genes can be mutated to produce dominant modifying effects on variegation. These mutations may be in dosage sensitive genes that encode chromosomal proteins. If these proteins assemble into multimolecular complexes (ZUCKERKANDL 1974; SINCLAIR, MOTTUS and GRIGLIATTI 1983; LOCKE, KOTARSKI and TARTOF 1988), changes in the concentration of any one component may determine the extent of the spreading of heterochromatin.

Recent molecular studies of two modifiers of PEV have provided support for the hypothesis that these genes encode chromosomal proteins. JAMES and ELGIN (1986) isolated an antibody that binds primarily to the chromocenter in salivary gland nuclei. The antigen recognized by this antibody is encoded by the *Su(var)205* gene (EISSENBERG *et al.* 1990) which was first identified by a dominant mutation that suppresses the PEV of euchromatic genes (SINCLAIR, MOTTUS and GRIGLIATTI 1983). A duplication of the chromosomal region including this gene suggests that the gene is dosage sensitive (LOCKE, KOTARSKI and TARTOF 1988). Another dosage sensitive modifier of PEV, *Suvar(3)7*, has also been cloned by REUTER *et al.* (1990). These investigators propose that the $Suvar(3)7^+$ protein binds to DNA and interacts with other proteins. While it is possible that many of the modifiers of PEV are similar to *Su(var)205* and *Suvar(3)7* in encoding chromosomal proteins, it is also possible that the modifiers act through a variety of molecular mechanisms.

Although several studies have described the effects of modifiers on the variegation of euchromatic genes (REUTER, WERNER and HOFFMANN 1982; SINCLAIR, MOTTUS and GRIGLIATTI 1983; LOCKE, KOTARSKI and TARTOF 1988), very little is known about the effect of these same modifiers on genes that are normally found in heterochromatin. One modifier, the *Y* chromosome, has reciprocal effects on the variegation of the heterochromatic *light* gene and the variegation of euchromatic genes (SCHULTZ 1936; BAKER and REIN 1962). Do *Su(var)* mutations also act in a reciprocal fashion on genes normally found in heterochromatin?

In a previous study we showed that one *Su(var)* enhances the variegation of two heterochromatic genes (HEARN *et al.* 1988; WAKIMOTO and HEARN 1990). To extend this analysis and determine if reciprocity is a general characteristic of *Su(var)* mutations, we surveyed the effects of fourteen modifiers of position effect on the variegation of the *light* gene. We show here that six mutations strongly enhance the variegation of the *light* gene in several rearrangements in contrast to the suppressive effects of these modifiers on variegating euchromatic genes. We have also tested the two *Su(var)*s with the strongest effects on the *light* gene for their effects on other genes found in 2L heterochromatin. Our results show that the *Su(var)205* mutation enhances the variegation of three 2Lh genes and *Su(var)208* mutation enhances the variegation of five of these genes.

MATERIALS AND METHODS

Drosophila stocks: The isolation and characterization of the *light*-variegated chromosome rearrangements (designated lt^x) are described in WAKIMOTO and HEARN (1990). The isolation and genetic properties of most of the *Su(var)* mutations are described in SINCLAIR, MOTTUS and GRIGLIATTI (1983). *Su(var)208* (2-5.7), *Su(var)307* (3-47.4), *Su(var)308* (3-49.2), *Su(var)310* (3-54.4) and *Su(var)321* (3-47.6) were isolated in a second screen (T. A. GRIGILATTI, unpublished data). All *Su(var)* mutations were recovered after EMS mutagenesis of the same *b lt rl* marker strain. The lethal alleles of the six genes located in 2L heterochromatin were identified by HILLIKER (1976). The cta^{WU31} mutation was isolated by SCHÜPBACH and WIESCHAUS (1989). All other mutations are described in LINDSLEY and GRELL (1968) or LINDSLEY and ZIMM (1985, 1986, 1987, 1990).

Cultures for eye pigment assays were maintained at 22° on cornmeal-sucrose medium with Tegosept added as mold inhibitor. Cultures for viability assays were maintained at 25° on cornmeal-molasses-brewers' yeast-Tegosept medium.

Pigment assays: Eye pigment extractions and assays were performed as described by SINCLAIR, MOTTUS and GRIGLIATTI (1983). Pigment levels were measured separately in males and females to account for any sexually dimorphic properties common among *Su(var)* mutants (SINCLAIR, MOTTUS and GRIGLIATTI 1983). Twenty-five females and twenty-five males of each genotype to be assayed were collected on the day of eclosion, aged 5–8 days and frozen at −70°. Eye pigments from five heads from each sex were extracted in 30 µl of 0.25 M β-mercaptoethanol in 1% NH_4OH. Pigment levels were measured fluorometrically using 5-µl aliquots from five separate extractions of each sex using a MPS-1 Zeiss microscope. These pigment values are presented in Tables 1 and 2 and Figure 3 as percentages of values obtained from wild-type (*Oregon-R*) individuals.

Flies that were heterozygous for a lt^x rearrangement and a hypomorphic allele of the *light* gene (lt^1) were used to assess the effects of the *Su(var)* mutations on the variegation of the *light* gene. Two trials of pigment assays were completed for most combinations of the lt^x rearrangements and the chromosome *2 Su(var)* mutations (*Su(var2)*). Males of the genotype $w^{m4};Su(var2)\ b\ lt^1rl/CyO$ were crossed to $+/+;lt^x/Gla$ (*CyO* and *Gla* are dominantly marked balancer chromosomes) or lt^{x13}/lt^{x13} virgin females. The pigment values of $lt^x/Su(var2)\ lt^1$ progeny were compared to those of progeny produced from a control cross using $Su(var2)^+$ fathers. Control lt^x pigment levels were measured separately for each trial (Table 1 and Figure 3).

TABLE 1

Effects of chromosome *2 Su(var)* mutations on *lt*-variegation

			Percent pigmentation[a]											
			lt^{x2}		lt^{x4}		lt^{x6}		lt^{x13}		lt^{x18}		lt^{x24}	
Su(var)	Trial	Sex	*Su(var)*[+]	*Su(var)*	*Su(var)*[+]	*Su(var)*	*Su(var)*[+]	*Su(var)*	*Su(var)*[+]	*Su(var)*	*Su(var)*[+]	*Su(var)*	*Su(var)*[+]	*Su(var)*
201	I	F	57.0 ± 5.5	28.6 ± 1.4*	88.2 ± 2.6	82.7 ± 5.2	50.1 ± 4.9	32.1 ± 1.8*	66.1 ± 5.2	68.4 ± 3.0	31.0 ± 3.6	29.2 ± 1.8	82.3 ± 5.7	66.7 ± 2.3
	II	F	47.0 ± 7.3	34.9 ± 3.7*	91.7 ± 4.6	72.2 ± 9.2*	42.1 ± 3.4	32.5 ± 1.7*	105.0 ± 7.1	81.2 ± 4.0*	ND		89.8 ± 4.0	71.9 ± 3.6•
	I	M	54.6 ± 10.9	29.1 ± 1.8*	118.7 ± 2.7	99.7 ± 2.8	64.1 ± 8.2	48.4 ± 2.3*	84.4 ± 4.7	84.5 ± 4.7	26.9 ± 1.4	28.9 ± 0.6	102.5 ± 2.5	96.1 ± 4.1
	II	M	46.2 ± 9.5	34.1 ± 6.9*	93.4 ± 9.6	83.2 ± 5.8*	53.3 ± 4.6	40.8 ± 3.5*	91.5 ± 7.1	86.7 ± 4.9	ND		77.5 ± 10.5	85.1 ± 8.8
206	I	F	57.0 ± 5.5	35.0 ± 2.3*	88.2 ± 2.6	79.5 ± 8.1*	50.1 ± 4.9	35.9 ± 3.4*	66.1 ± 5.2	65.0 ± 0.8	31.0 ± 3.6	20.6 ± 2.1*	82.3 ± 5.7	68.3 ± 5.8
	II	F	38.5 ± 2.0	39.8 ± 4.2	70.6 ± 2.9	68.6 ± 4.8	41.8 ± 3.5	42.4 ± 2.2	70.7 ± 0.9	68.5 ± 3.1	40.9 ± 6.0	33.5 ± 1.3*	76.0 ± 2.7	60.1 ± 4.7*
	I	M	54.6 ± 10.9	28.5 ± 1.7*	118.7 ± 2.7	106.0 ± 4.5	64.1 ± 8.2	56.3 ± 6.3	84.4 ± 4.7	84.8 ± 5.0	26.9 ± 1.4	21.7 ± 3.1	102.5 ± 2.5	94.9 ± 7.8
	II	M	45.2 ± 3.7	38.0 ± 3.2*	77.1 ± 6.3	68.5 ± 4.9*	54.1 ± 8.1	45.4 ± 1.4*	80.0 ± 4.7	70.9 ± 5.9	31.6 ± 1.9	34.6 ± 1.3	81.8 ± 3.3	65.2 ± 5.5*
207	I	F	57.0 ± 5.5	43.5 ± 2.4*	88.2 ± 2.6	81.0 ± 6.6	50.1 ± 4.9	32.7 ± 0.7*	66.1 ± 5.2	67.6 ± 2.8	31.0 ± 3.6	24.7 ± 0.6*	82.3 ± 5.7	69.5 ± 3.4
	II	F	47.0 ± 7.3	28.6 ± 2.0*	91.7 ± 4.6	80.3 ± 5.1*	42.1 ± 3.4	37.1 ± 2.3*	105.0 ± 7.1	82.5 ± 3.9*	ND		89.8 ± 4.0	79.5 ± 7.1*
	I	M	54.6 ± 10.9	43.2 ± 3.0*	118.7 ± 2.7	109.6 ± 8.8	64.1 ± 8.2	51.3 ± 6.7*	84.4 ± 4.7	93.9 ± 3.0*	26.9 ± 1.4	22.1 ± 3.9*	102.5 ± 2.5	110.3 ± 4.4
	II	M	46.2 ± 9.5	35.6 ± 4.7*	93.4 ± 9.6	83.5 ± 5.4*	53.3 ± 4.6	46.2 ± 10.5	91.5 ± 7.1	91.8 ± 2.3	ND		77.5 ± 10.5	96.1 ± 3.1
210	I	F	57.0 ± 5.5	51.0 ± 2.2*	88.2 ± 2.6	72.5 ± 1.5*	50.1 ± 4.9	35.5 ± 2.4*	66.1 ± 5.2	64.9 ± 4.7	31.0 ± 3.6	35.0 ± 4.4	82.3 ± 5.7	71.7 ± 0.9
	II	F	38.5 ± 2.0	41.9 ± 3.1	70.6 ± 2.9	75.6 ± 3.6	41.8 ± 3.5	45.2 ± 5.4	70.7 ± 0.9	72.6 ± 3.4	40.9 ± 6.0	34.5 ± 1.3*	ND	
	I	M	54.6 ± 10.9	57.8 ± 4.2	118.7 ± 2.7	89.2 ± 2.4*	64.1 ± 8.2	52.4 ± 8.2*	84.4 ± 4.7	74.9 ± 6.2*	26.9 ± 1.4	38.7 ± 1.2*	102.5 ± 2.5	85.4 ± 1.9
	II	M	45.2 ± 3.7	50.6 ± 6.2	77.1 ± 6.3	83.1 ± 6.0	54.1 ± 8.1	56.9 ± 6.2	80.0 ± 4.7	73.8 ± 9.0	31.6 ± 1.9	40.5 ± 4.8*	ND	
214	I	F	57.0 ± 5.5	37.0 ± 1.5*	88.2 ± 2.6	91.6 ± 5.1	50.1 ± 4.9	41.0 ± 2.2*	66.1 ± 5.2	77.0 ± 3.4*	31.0 ± 3.6	24.5 ± 2.8*	82.3 ± 5.7	81.6 ± 3.1
	II	F	47.0 ± 7.3	44.8 ± 2.4	91.7 ± 4.6	88.7 ± 3.4	42.1 ± 3.4	43.1 ± 5.4	105.0 ± 7.1	93.7 ± 2.3*	24.6 ± 3.9	40.6 ± 3.6*	89.8 ± 4.0	93.7 ± 2.3
	I	M	54.6 ± 10.9	41.7 ± 3.2*	118.7 ± 2.7	117.8 ± 3.2	64.1 ± 8.2	63.9 ± 3.5	84.4 ± 4.7	101.3 ± 2.1	26.9 ± 1.4	27.3 ± 2.2	102.5 ± 2.5	113.4 ± 9.1
	II	M	46.2 ± 9.5	49.3 ± 6.2	93.4 ± 9.6	92.5 ± 1.4	53.3 ± 4.6	52.1 ± 8.3	91.5 ± 7.1	94.6 ± 1.5	26.7 ± 2.5	37.9 ± 3.9*	77.5 ± 10.5	97.6 ± 1.3*
216	I	F	57.0 ± 5.5	37.1 ± 2.5*	88.2 ± 2.6	76.4 ± 3.9*	50.1 ± 4.9	34.4 ± 2.6*	66.1 ± 5.2	68.6 ± 0.7	31.0 ± 3.6	22.5 ± 3.5*	82.3 ± 5.7	68.0 ± 3.1
	II	F	38.5 ± 2.0	31.4 ± 1.4*	70.6 ± 2.9	64.0 ± 3.3*	41.8 ± 3.5	36.3 ± 3.2*	70.7 ± 0.9	61.7 ± 6.8*	40.9 ± 6.0	32.9 ± 0.6*	76.0 ± 2.7	72.8 ± 4.1
	I	M	54.6 ± 10.9	35.7 ± 2.6*	118.7 ± 2.7	90.9 ± 6.8*	64.1 ± 8.2	47.0 ± 0.9*	84.4 ± 4.7	80.7 ± 2.7	26.9 ± 1.4	28.9 ± 4.9	102.5 ± 2.5	83.9 ± 3.1
	II	M	ND		77.1 ± 6.3	67.5 ± 6.2*	54.1 ± 8.1	48.1 ± 7.5	80.0 ± 4.7	67.0 ± 7.0*	31.6 ± 1.9	37.4 ± 2.9*	81.8 ± 3.3	83.9 ± 2.9

[a] Pigment levels are expressed as a percentage of the pigment levels of a lt^+ *Oregon-R* strain. The mean ± SEM of five assays is shown. ND = Not determined. * $P \leq 0.05$.

TABLE 2

Effect of chromosome *3 Su(var)* mutations on *lt*-variegation

		lt^{x2}		lt^{x6}		lt^{x13}	
Su(var)	Sex	*Su(var)*$^+$	*Su(var)*	*Su(var)*$^+$	*Su(var)*	*Su(var)*$^+$	*Su(var)*
307	F	25.0 ± 1.7	24.4 ± 1.4	30.3 ± 0.7	31.4 ± 1.3	57.9 ± 4.2	63.0 ± 2.6
	M	32.4 ± 5.0	29.3 ± 6.1	43.6 ± 3.8	36.3 ± 7.9	73.9 ± 6.2	70.5 ± 4.4
308	F	25.3 ± 0.8	30.1 ± 3.4*	24.2 ± 2.5	27.8 ± 1.8*	58.7 ± 2.4	62.1 ± 1.8*
	M	36.6 ± 4.8	33.0 ± 4.8*	38.2 ± 3.9	32.6 ± 2.9*	76.5 ± 3.4	74.8 ± 5.0
310	F	29.9 ± 2.4	33.1 ± 5.1	30.0 ± 1.3	34.6 ± 6.0	59.7 ± 5.6	63.9 ± 4.7
	M	35.2 ± 7.7	33.0 ± 2.4	47.4 ± 1.7	49.8 ± 6.0	82.5 ± 6.6	83.4 ± 5.8
316	F	29.8 ± 2.9	30.8 ± 3.0	21.8 ± 2.9	24.6 ± 0.8*	61.6 ± 2.7	59.3 ± 4.8
	M	36.3 ± 3.1	28.7 ± 1.3*	43.1 ± 4.4	39.9 ± 3.8	68.0 ± 1.1	60.8 ± 8.5*
319	F	36.7 ± 5.3	30.1 ± 1.9*	32.3 ± 2.8	35.2 ± 1.0	57.2 ± 6.1	70.2 ± 3.0*
	M	27.7 ± 2.7	23.9 ± 2.3	41.3 ± 2.3	36.2 ± 3.7*	80.3 ± 5.5	76.9 ± 6.1
321	F	31.6 ± 4.3	30.3 ± 3.9	39.7 ± 8.6	33.5 ± 2.0	71.3 ± 3.9	70.5 ± 2.5
	M	37.3 ± 3.4	35.7 ± 2.0	43.6 ± 5.5	36.6 ± 2.9*	82.8 ± 2.8	73.7 ± 5.4

[a] All symbols are the same as described in Table 1.

Three of the lt^x rearrangements used in our analysis of the *Su(var2)* mutations were chosen for analysis of chromosome *3 Su(var)* mutations (*Su(var3)*). Pigment data for these crosses are shown in Table 2. lt^x/Gla or lt^{x13}/lt^{x13} virgin females were crossed to *b* $lt^1rl/Tft;Su(var3)/Ly$ males (*Tft* is a dominant second chromosome marker and *Ly* is a dominant third chromosome marker). Pigment values of the F_1 $lt^x/lt^1;Su(var3)/+$ progeny and control $lt^x/lt^1;Ly/+$ siblings are shown in Table 2. Differences between these values indicate effects of the *Su(var)* mutation.

Standard statistical techniques were applied to determine significant changes from basal *lt* variegating levels due to *Su(var)* action. *Su(var2)* values were compared to control values by ANOVA followed by Dunnett's multiple range test (ZAR 1984). Differences in pigment values from *Su(var3)* progeny and their *Su(var3)*$^+$ siblings were evaluated using unpaired *t*-tests. This eliminates inter-trial error and minimizes variability due to the assay system. Although differences in the effects of particular *Su(var)*s on males and females were seen in several cases, these differences were not consistent between trials for *Su(var2)* mutations and were small (low *t*-values) for all *Su(var3)* mutations tested.

Complementation tests with the genes in 2L heterochromatin: Recombinant chromosomes were selected for each combination of a lethal allele of each 2Lh gene (*l(2) EMS*) and a *Su(var2)* mutation and then balanced over $SM1,Cy,lt^H$. This *SM1* balancer chromosome, which carries an EMS-induced *lt* mutation, was recovered by A. HILLIKER and kindly provided by D. HOLM. All recombinant chromosomes were obtained at the appropriate frequency given the map positions of *Su(var)205* and *Su(var)208* and retained strong suppressive effects on w^{m4}.

To measure viability, several sets of ten *Su(var) l(2)EMS/SM1* females were mated to ten lt^x/Gla or lt^{x13}/lt^{x13} males in half-pint milk bottles. The flies were subcultured into new bottles after 5 days and adults were discarded after 5 more days. For each bottle, the recovery of the class of progeny heterozygous for the *l(2)EMS* mutation and the lt^x rearrangement was compared to the recovery of $lt^x/SM1$ siblings. The ratio of these classes was determined separately for each sex. In most cases, each subculture was treated as a separate trial. The mean viability ratio and the standard error of the mean (SEM) for all trials of each cross are shown in Tables 3 and 4. The goodness of fit hypothesis that there was no effect of the *Su(var)* mutation on viability was evaluated by using the *G*-test of independence to compare the progeny counts obtained in each set of *Su(var)* and *Su(var)*$^+$ crosses. In 3 of the 16 cases we observed a significant difference in the viability of *Su(var)* and *Su(var)*$^+$ flies in the control crosses. Hence, the viabilities of *Su(var)208 EMS56-8/*lt^x females and *Su(var)208 EMS40-5/*lt^x females and males were evaluated using *G*-tests that took the differences in the control crosses (data not shown) into account. The MLIKELY computer program (kindly provided by LEONARD ROBBINS) was used to evaluate the counts from each set of four crosses. Sets of crosses that were determined to be significantly different ($P < 0.05$) are denoted by asterisks in Table 3 and 4.

To assay for the effect of *Su(var)* mutations on the variegation of *concertina* (*cta*), a maternal effect gene in 2Lh, we selected *Su(var)205* cta^{WU31} and *Su(var)208* cta^{WU31} recombinant chromosomes. The total number of progeny produced by cta^{WU31}/lt^x females was compared to the total number of progeny produced by *Su(var)* cta^{WU31}/lt^x females for each lt^x rearrangement tested. Virgin females were collected and aged 3 days. Individual females were mated to two wild-type *Canton-S* (*CS*) males, eggs were collected for 5 days and the progeny were counted through day 18. The mean ± SEM of the total number of progeny per female for two to five trials of each cross is presented in Table 5. There was no significant difference between the total number of progeny produced by cta^{WU31}/CS and *Su(var)208* cta^{WU31}/CS females. Thus, we have compared directly the mean of total number of progeny produced by *Su(var)208* cta^{WU31}/lt^x females to that of cta^{WU31}/lt^x females and used *t*-tests to determine whether these values were significantly different (Table 5).

RESULTS

Properties of *light* variegating rearrangements: The *light* gene (*lt*) is an essential gene and is required

TABLE 3

The effect of *Su(var)205* on the variegation of 2Lh genes

2Lh gene	Parental genotype[a] Maternal	Parental genotype[a] Paternal		*Su(var)*+ n^b	*Su(var)*+ Viability[c]	*Su(var)205* n	*Su(var)205* Viability
40Fa	*EMS 56-8*	lt^{x3}	F	1549 (13)[d]	0.98 ± 0.090	1289 (8)	1.09 ± 0.075
			M	1579	1.17 ± 0.081	1300	1.07 ± 0.069
		lt^{x13}	F	1490 (20)	1.01 ± 0.081	771 (8)	0.85 ± 0.049
			M	1437	1.07 ± 0.050	580	0.88 ± 0.061*
		lt^{x21}	F	975 (9)	1.08 ± 0.054	1202 (9)	0.89 ± 0.039
			M	986	0.94 ± 0.101	1163	1.25 ± 0.132*
		lt^{x23}	F	1389 (13)	0.98 ± 0.079	383 (5)	0.32 ± 0.054*
			M	1168	1.10 ± 0.112	354	0.64 ± 0.076*
40Ff	*EMS 56-4*	lt^{x3}	F	1563 (10)	0.74 ± 0.067	560 (3)	0.38 ± 0.066*
			M	1626	0.56 ± 0.059	550	0.53 ± 0.121
		lt^{x13}	F	791 (6)	0.84 ± 0.061	1095 (10)	0.65 ± 0.088*
			M	600	0.54 ± 0.055	988	0.75 ± 0.049*
		lt^{x21}	F	1449 (13)	0.86 ± 0.065	304 (5)	0.77 ± 0.152
			M	1404	0.66 ± 0.059	326	0.66 ± 0.051

[a] 2Lh lethal/Balancer females were crossed to lt^x/Balancer or for lt^{x13} to homozygous lt^{x13} males.
[b] Total progeny for all trials.
[c] Viability is determined by dividing the total number of 2Lh lethal/lt^x individuals by the total number of Balancer/lt^x individuals for each trial. The mean ± SEM of the viabilities from all trials of each cross is shown.
[d] Number of trials. * Significantly different from *Su(var)*+ crosses (*G*-test; $P \leq 0.05$).

for wild-type levels of eye pigmentation. It is located within or very near the centromeric heterochromatin of chromosome *2*. Rearrangements that variegate for *lt* displace the gene to distal euchromatin and result in mottled eye color phenotypes (HESSLER 1958; WAKIMOTO and HEARN 1990). We took advantage of the mutant eye phenotype and used pigment assays to provide a rapid and sensitive means to measure the effects of 14 dominant modifiers (designated *Su(var)*s) on *lt* variegation.

Six different rearrangements that variegate for *lt* were used in these studies. They were tested in heterozygous combination with the hypomorphic lt^1 mutation, in the absence (*Su(var)*+) or the presence (*Su(var)*) of modifiers. These rearrangements (designated lt^x) differ in the severity of their effects on the displaced lt^+ gene. Thus, they provided a broad phenotypic range of sensitivity to determine whether a modifier causes suppression (increased pigmentation), enhancement (decreased pigmentation) or has no effect on variegation. In addition, they allowed us to assess whether the position of the heterochromatic breakpoint is important in determining sensitivity to a given modifier. An estimate of the position of the heterochromatic breakpoints of these rearrangements has been determined cytologically (WAKIMOTO and HEARN 1990) (Figure 1). Some breakpoints lie more proximally and move a large region of heterochromatin (the h35, h36 and h37 regions of the mitotic map) along with the lt^+ gene to distal euchromatin. Others are broken within the most distal block of heterochromatin (h35), moving a smaller block of heterochromatin along with the lt^+ gene. In general, rearrangements broken in h35 have a more extreme variegated phenotype.

The basal level of pigmentation was determined for lt^1/lt^1 homozygotes and lt^x/lt^1 heterozygotes and expressed as a percentage of the levels of wild-type *Oregon-R* flies. Measurements of the pigment levels of lt^1 male and female homozygotes were very consistent, ranging from 30.3 ± 1.2% to 34.2 ± 1.0% of wild type in three separate trials. As expected, the pigment levels of the strains carrying *lt*-variegating rearrangements were more variable. As shown in Table 1, the mean pigment levels for some lt^x strains were often different between trials. lt^{x18}/lt^1 flies had pigment levels similar to lt^1 homozygotes indicating that the lt^{x18} rearrangement inactivates the lt^+ gene in most if not all of the ommatidia. Suppression of variegation by a *Su(var)* mutation should have been detectable using this strain since increases in pigment would have been easily assayed. Flies of the genotypes lt^{x2}/lt^1 and lt^{x6}/lt^1 produced 40–60% of wild-type pigment levels. Suppression or enhancement should have been detected in strains carrying the lt^{x2} and lt^{x6} rearrangements, since these had moderate effects when heterozygous with lt^1. Finally, the lt^{x4}, lt^{x13} and lt^{x24} rearrangements produced weak but visible effects on variegation and when heterozygous with lt^1, flies carrying these rearrangements produced 70–100% of wild-type pigment levels. These rearrangements should have permitted detection of enhancing effects with modifiers.

TABLE 4

The effect of *Su(var)208* on the variegation of 2Lh genes

	Parental genotype[a]			*Su(var)*[+]		*Su(var)208*	
2Lh gene	Maternal	Paternal		n^b	Viability[c]	n	Viability
40Fa	*EMS 56-8*	lt^{x3}	F	1549 (13)[d]	0.98 ± 0.090	1024 (12)	0.77 ± 0.051[e]
			M	1579	1.17 ± 0.081	948	0.95 ± 0.092*
		lt^{x13}	F	1490 (20)	1.01 ± 0.081	653 (12)	1.03 ± 0.116[e]*
			M	1437	1.07 ± 0.050	566	0.95 ± 0.097
		lt^{x23}	F	1389 (13)	0.98 ± 0.079	429 (8)	0.51 ± 0.074[e]
			M	1168	1.10 ± 0.112	378	0.64 ± 0.095*
40Fc	*EMS40-2*	lt^{x3}	F	783 (10)	1.07 ± 0.141	484 (10)	0.90 ± 0.122
			M	765	1.31 ± 0.133	525	0.61 ± 0.093*
		lt^{x13}	F	467 (4)	1.18 ± 0.088	458 (4)	0.65 ± 0.054*
			M	504	1.06 ± 0.052	299	0.22 ± 0.042*
		lt^{x21}	F	300 (6)	1.25 ± 0.063	143 (8)	0.67 ± 0.149*
			M	307	0.97 ± 0.149	147	0.43 ± 0.094*
		lt^{x23}	F	843 (12)	0.98 ± 0.095	851 (14)	0.48 ± 0.056*
			M	719	0.75 ± 0.060	700	0.24 ± 0.033*
40Fd	*EMS 40-7*	lt^{x3}	F	494 (4)	1.36 ± 0.108	453 (4)	0.98 ± 0.086
			M	473	1.15 ± 0.108	453	0.85 ± 0.130
		lt^{x23}	F	525 (5)	1.07 ± 0.102	417 (5)	0.77 ± 0.071
			M	426	0.90 ± 0.143	348	0.90 ± 0.061
40Ff	*EMS56-4*	lt^{x4}	F	1209 (12)	0.74 ± 0.080	1070 (12)	0.36 ± 0.054*
			M	1242	0.48 ± 0.047	951	0.13 ± 0.038*
		lt^{x6}	F	421 (4)	1.23 ± 0.099	260 (4)	1.11 ± 0.209
			M	510	0.99 ± 0.045	315	0.76 ± 0.117
		lt^{x11}	F	568 (5)	1.21 ± 0.135	495 (5)	1.41 ± 0.174
			M	580	1.36 ± 0.305	535	1.24 ± 0.177
		lt^{x21}	F	1449 (13)	0.86 ± 0.065	591 (8)	0.48 ± 0.051*
			M	1404	0.66 ± 0.059	648	0.55 ± 0.052
		lt^{x24}	F	742 (4)	1.00 ± 0.062	524 (4)	0.85 ± 0.096
			M	702	0.82 ± 0.084	451	0.48 ± 0.053*
40Fe	*EMS 56-24*	lt^{x3}	F	832 (6)	0.82 ± 0.084	636 (6)	1.18 ± 0.169*
			M	868	0.85 ± 0.083	554	1.18 ± 0.185
		lt^{x13}	F	542 (6)	1.25 ± 0.132	464 (8)	1.14 ± 0.167
			M	487	1.23 ± 0.160	405	1.14 ± 0.112
		lt^{x21}	F	280 (5)	1.13 ± 0.057	294 (5)	1.26 ± 0.171
			M	306	0.88 ± 0.126	312	0.79 ± 0.123
		lt^{x23}	F	933 (10)	1.07 ± 0.079	703 (10)	1.12 ± 0.087
			M	799	1.03 ± 0.082	590	1.27 ± 0.091
40Fg	*EMS 40-5*	lt^{x3}	F	326 (6)	1.44 ± 0.085	268 (6)	1.26 ± 0.235[e]
			M	356	1.43 ± 0.095	315	0.93 ± 0.117[e]
		lt^{x13}	F	480 (5)	1.22 ± 0.098	401 (5)	1.09 ± 0.093[e]
			M	429	1.31 ± 0.087	414	0.97 ± 0.051[e]
		lt^{x23}	F	719 (9)	1.13 ± 0.092	584 (10)	1.17 ± 0.143[e]
			M	650	1.15 ± 0.175	496	1.04 ± 0.141[e]

[a-d] All symbols are the same as those used in Table 3.

[e] The viabilities from the *Canton-S* control crosses for these sets of crosses were shown to be significantly different. We therefore evaluated the difference between *Su(var)* and *Su(var)*[+] crosses by using *G*-tests that incorporated the *Canton-S* control crosses (see MATERIALS AND METHODS).

Some *Su(var)* mutations enhance *lt*-variegation: The 14 *Su(var)* mutations used in this study were isolated and mapped by SINCLAIR, MOTTUS and GRIGLIATTI (1983) and T. A. GRIGLIATTI (unpublished data). All of these mutations were originally isolated as strong, dominant suppressors of variegation of the *white*[+] gene on the *In(1)w*^{m4} inversion and they have been shown to also suppress the variegation of at least

TABLE 5

The effect of *Su(var)208* on the variegation of the *cta*• gene

Su(var)208 crosses[a]	Mean progeny/female per trial: I	II	III	IV	V	Ratio of progeny[b] 208 *cta/cta*
cta^{WU31}/lt^{x3}	36.4 ± 2.95 (14),[c]	47.0 ± 2.28 (6),	35.2 ± 6.33 (11)			
$Su(var)208\ cta^{WU31}/lt^{x3}$	28.9 ± 2.49 (10),	18.3 ± 3.95 (8),	37.6 ± 6.55 (9)			μ = 0.75 ± 0.197 (P = 0.169)[d]
cta^{WU31}/lt^{x13}	51.0 ± 3.35 (27),	32.8 ± 4.55 (12),	44.0 ± 2.67 (12),	38.5 ± 3.52 (15)		
$Su(var)208\ cta^{WU31}/lt^{x13}$	30.4 ± 3.12 (35),	32.2 ± 2.81 (12),	26.9 ± 4.05 (10),	31.9 ± 3.17 (14)		μ = 0.75 ± 0.093 (P = 0.033)
cta^{WU31}/lt^{x23}	37.3 ± 2.43 (9),	40.8 ± 3.12 (18)				
$Su(var)208\ cta^{WU31}/lt^{x23}$	13.5 ± 4.14 (6),	15.8 ± 3.49 (14)				μ = 0.38 ± 0.013 (P = 0.007)
cta^{WU31}/CS	56.6 ± 5.61 (12),	71.4 ± 3.99 (20),	88.2 ± 6.24 (5),	53.8 ± 2.73 (23),	46.1 ± 4.79 (12)	
$Su(var)208\ cta^{WU31}/CS$	43.3 ± 5.91 (12),	71.8 ± 3.99 (18),	85.2 ± 10.98 (5),	55.9 ± 3.48 (17),	51.1 ± 3.41 (7)	μ = 0.98 ± 0.058 (P = 0.873)

[a] Individual females of the genotype listed were crossed to two wild-type *Canton-S (CS)* males.
[b] The ratio of mean progeny per *Su(var)208 cta/lt*^{x} female to mean progeny per *cta/lt*^{x} female was determined for each trial. μ is the mean ± SEM of these ratios.
[c] Mean ± SEM for each trial (the number of females tested for each trial).
[d] The ratios from *Su(var)208* trials were compared to the ratios from *Su(var)*$^+$ trials using the *t*-test.

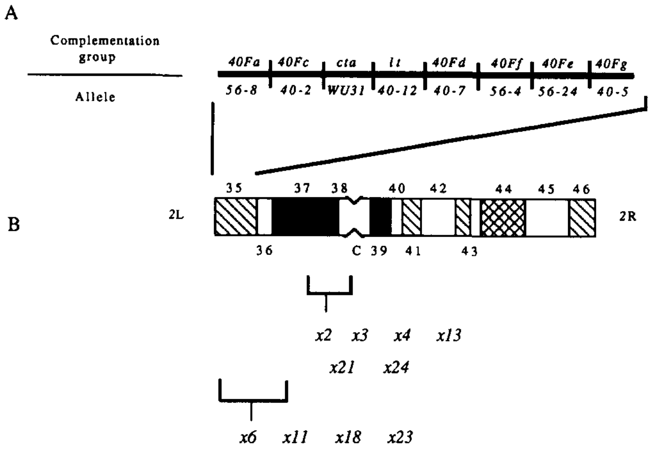

FIGURE 1.—Summary of the genetic map of 2Lh and the cytogenetic map of chromosome *2* heterochromatin. (A) Diagram showing the proximal to distal order of the genes that have been mapped to 2L heterochromatin (HILLIKER 1976; WAKIMOTO and HEARN 1990). The mutant alleles used in this study are listed below each gene. (B) The standard mitotic map of the heterochromatin of chromosome *2* showing the position of the heterochromatic breakpoints of the rearrangements used in this study. Breakpoints were mapped by WAKIMOTO and HEARN (1990) using Hoechst 33258 alone. The map showing the banding pattern of Hoechst and N-banding was taken from PIMPINELLI and DIMITRI (1989). DIMITRI (1991) has cytologically mapped the 2Lh genes within or just distal to band h35.

two other euchromatic genes (the *Stubble* mutation and $brown^+$ gene). The locations of the modifiers on the genetic map are shown in Figure 2. They have been grouped into two categories, clustered and nonclustered. Clusters refer to modifiers that map within a 3-cM interval. Recent complementation analysis suggests that the 2L cluster contains at least two complementation groups (T. A. GRIGLIATTI, unpublished data). Thus, we have tested a minimum of six separate chromosome *2* and two separate chromosome *3* modifier loci.

The effects of eight chromosome *2* modifiers on *lt* variegation are summarized by Table 1 and Figure 3. Because of the inter-trial variability in pigment levels measured in lt^x flies, pigment differences between the *Su(var)* and $Su(var)^+$ classes were considered biologically significant only if two criteria were met: (1) both trials gave statistically significant differences and (2) the direction of the change (*i.e.*, enhancement or suppression) was the same for both trials.

Two of the chromosome *2 Su(var)* mutations, *Su(var)210* and *214* did not appear to enhance *lt* variegation. Six mutations were classified as enhancers of variegation. We consider four of these, *Su(var)201, 206, 207* and *216*, to be moderate enhancers because they significantly enhanced the variegation of *lt* on at least two rearrangements. Each mutation caused reductions in pigment levels ranging from 5 to 30 percentage units below control $Su(var)^+$ levels. Two mutations, *Su(var) 205* and *208*, which map outside of the 2L cluster, had stronger effects that were more consistent between trials. The effects of these two modifiers are presented graphically in Figure 3. With the exception of lt^{x18}, all rearrangements were significantly affected by *Su(var)205* and *Su(var)208* in both sexes. The enhancing effects were easily detected visually and resulted in drops of at least 15 percentage units compared to the pigment levels in the $Su(var)^+$ control classes. The low basal levels of pigmentation of lt^{x18}/lt^1 flies may have prevented the detection of any enhancing effect that the strong modifiers had on the lt^+ gene in this rearrangement.

In summary, the data in Table 1 and Figure 3 lead us to the two following general conclusions. First, all the lt^x rearrangements, including lt^{x18}, were susceptible to enhancement of variegation by some of the *Su(var2)* mutations. lt^{x2} and lt^{x6} were the most sensitive, since both were enhanced by at least five of the *Su(var2)* mutations. Second, we conclude that enhancement of *light* variegation is a general property of the *Su(var2)* mutations. Six mutations, representing six separate modifier loci, act as enhancers of *lt* variegation. Each of these *Su(var2)* mutations affected at least two different lt^x rearrangements. *Su(var)205* and *208* had the strongest and most consistent effects, enhancing *lt* variegation on all lt^x rearrangements except lt^{x18}. Suppression of variegation was rare; only

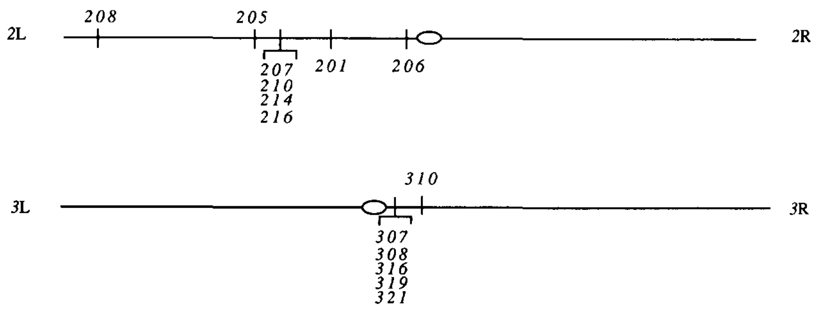

FIGURE 2.—The position on the genetic map of the *Su(var)* mutations used in this study. The data are taken from SINCLAIR, MOTTUS and GRIGLIATTI (1983) and from unpublished results (T. A. GRIGLIATTI). Mutations that are shown under the brackets were mapped to within 3 cM of each other on the left arm of chromosome *2* (2L) or the right arm of chromosome *3* (3R). The 2L cluster consists of at least two complementation groups, one is defined by the *Su(var)216* mutation.

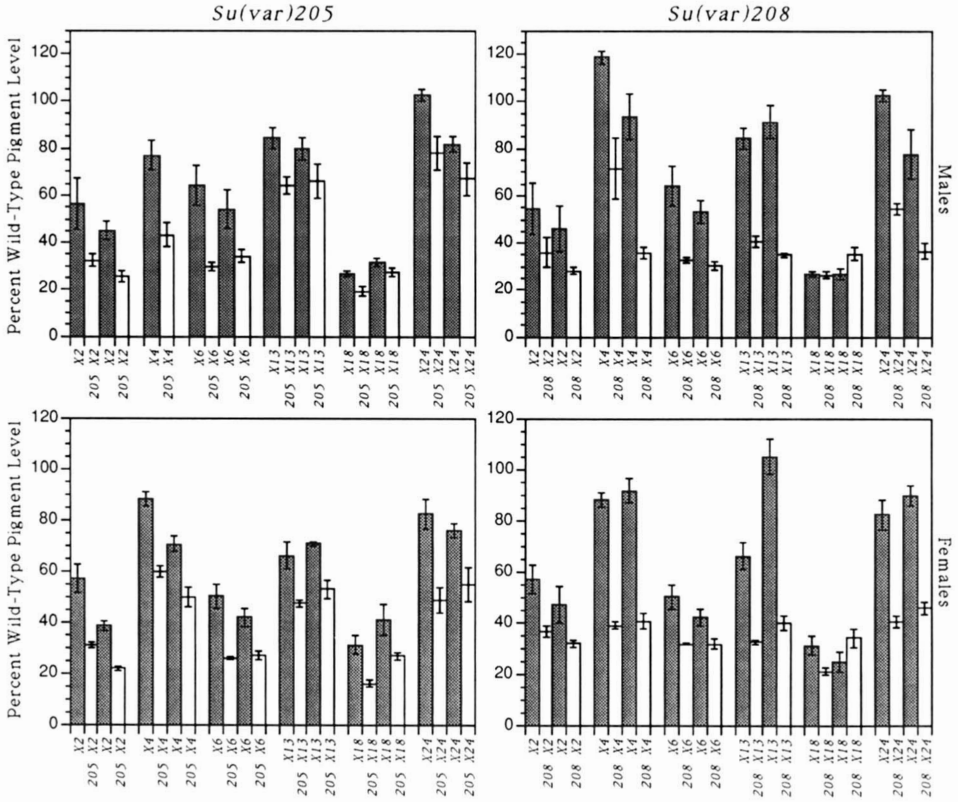

FIGURE 3.—A summary of the effects of *Su(var)205* and *208* on *light* variegation. Eye pigments were extracted from lt^x/lt^1 males and females that carried a wild-type or mutant allele of each *Su(var)* mutation. In most cases two trials for each genotype were carried out. Pigment values are presented as percent of wild type (*Oregon-R*) and error bars represent 95% confidence intervals [estimated as ±1.96 (SE)]. All of the $Su(var)/lt^x$ pigment levels were significantly different ($P < 0.05$) than the control $Su(var)^+/lt^x$ pigment levels with the exception of $Su(var)205/lt^{x18}$ males and $Su(var)208/lt^{x18}$ males and females.

a single case was observed. *Su(var)210* moderately increased pigment levels in lt^{x18} males.

The results of tests with the chromosome *3* modifiers are summarized in Table 2. These data are more limited than the data described above since each *Su(var3)* mutation was tested with only three lt^x rearrangements, and only a single trial was performed for each genotype. Nonetheless, the results showed that the *Su(var3)* mutations exhibited weak effects compared to the *Su(var2)* mutations, even when tested with the lt^{x2} and lt^{x6} rearrangements. None of the mutations resulted in pigment drops that exceeded 8% and, with the exception of *Su(var)319*, none of the mutations resulted in a greater than 5% increase in pigment. *Su(var)319* moderately suppressed *lt* variegation in lt^{x13}/lt^1 females. It did not, however, significantly affect either lt^{x2} or lt^{x6} which were the rearrangements most sensitive to the effects of the *Su(var2)* mutations. Evidence for possible suppressive effects by a *Su(var)* mutation was observed with *Su(var)308*. This mutation appeared to weakly suppress *lt* variegation in lt^{x2}/lt^1, lt^{x6}/lt^1 and lt^{x13}/lt^1 females. However, the effects were small in each case and males from these same crosses showed either weak enhancement or no effects of the modifier. We conclude from these data that none of the *Su(var3)* mutations act as strong suppressors or enhancers of *lt* variegation.

The *Su(var)205* and *208* mutations enhance the variegation of other heterochromatic genes: Eight genes including *light* have been mapped to 2L heterochromatin (2Lh) by HILLIKER (1976) and SCHÜPBACH and WIESCHAUS (1989) (Figure 1). All are represented by lethal mutations except the *concertina* gene, a maternal effect gene which when mutated can result in complete female sterility. To determine whether the *Su(var)* mutations that act as enhancers of lt^+ variegation have general effects on heterochromatic genes, we tested *Su(var)205* and *208*, the two strongest enhancers for their effects on the variegation of other 2Lh genes.

For these tests, crosses were made to generate individuals heterozygous for a lt^x rearrangement and a chromosome *2* carrying both a *Su(var)* mutation and a lethal allele of one of the 2Lh genes. The viability of these individuals was compared to the viability of control siblings and $lt^x/Su(var)^+$ *l(2Lh)EMS* individuals, generated in a second cross. The lt^x rearrangements differ in their effects on genes adjacent to *lt*. For example, the *40Fa* and *light* genes show variegated expression in the lt^{x13} rearrangement; other rearrangements such as lt^{x23} exhibit variegation of three of the 2Lh genes (WAKIMOTO and HEARN 1990). To determine if the modifiers affected the variegation of the 2Lh genes near *lt*, we chose at least two lt^x rearrangements for each gene assayed. The choice of the lt^x depended upon the predicted sensitivity to enhancement or suppression based on our previous complementation studies.

Su(var)205 was tested for its effects on the variegation of the essential genes, *40Fa* and *40Ff* (Table 3), and the maternal effect gene, *cta*. *Su(var)208* was tested with seven of the 2Lh genes (Tables 4 and 5).

The *40Fa* gene is the most distal of the 2Lh gene identified so far (HILLIKER 1976) and appears to be among the most sensitive to PEV. All the lt^x rearrangements that variegate for *lt* also show variegated *40Fa* expression (WAKIMOTO and HEARN 1990). The variegation of *40Fa* is detected as a variable roughened eye phenotype and a reduction in the viability of flies heterozygous for lt^x rearrangements and the *EMS 56-8* mutation. Both *Su(var)205* and *208* enhance these mutant phenotypes. *Su(var)205 EMS 56-8* and *Su(var)208 EMS 56-8* flies heterozygous for lt^{x3} or lt^{x23} exhibited the roughened eye phenotype at a frequency at least fourfold greater than their $Su(var)^+$ controls. In addition, both *Su(var)*s had the greatest effects on the lt^{x23} rearrangement; the viability of *Su(var)205 EMS 56-8*/lt^{x23} progeny and *Su(var)208 EMS 56-8*/lt^{x23} males was significantly lower than the $Su(var)^+$ controls (Tables 3 and 4). The difference in the severity of the effects observed with lt^{x23} compared to lt^{x3}, lt^{x13} and lt^{x21} may be due to differences in the position of the heterochromatic breakpoints. In lt^{x23}, only the distal-most block of heterochromatin, h35, is displaced, while in the three other rearrangements the breakpoints are more proximal, moving the bulk of 2L heterochromatin along with the variegating genes.

The data shown in Table 4 allow us to conclude that variegation of *40Fc* and *40Ff* was enhanced by *Su(var)208* in at least two different lt^x rearrangements in each case. Variegation of the *40Ff* gene in the lt^{x3} and lt^{x13} rearrangements was also enhanced by *Su(var)205* in females (Table 3). We have no evidence that the *40Fd* gene was enhanced by *Su(var)208*.

Four cases of suppression were also seen. *Su(var)205 EMS 56-8*/lt^{x21} males, *Su(var)205 EMS 56-4*/lt^{x13} males and *Su(var)208 EMS 56-24*/lt^{x3} females showed a statistically significant increase in viability relative to $Su(var)^+$ controls. The viability of *Su(var)208 EMS 56-8*/lt^{x13} females was also shown to be significantly greater than *EMS 56-8*/lt^{x13} females when the viabilities were compared using a *G*-test that accounts for the reduced viability seen in *Su(var)208 EMS 56-8/Canton-S* females relative to *EMS 56-8/Canton-S* females (data not shown). Since suppression was seen with only one rearrangement and in only one sex, we conclude that *Su(var)205 and Su(var)208* do not act as general suppressors of the variegation of heterochromatic genes. It is possible that suppression of the variegation of heterochromatic genes was due to additional modifiers in the genetic background, but we consider this hypothesis unlikely since no consistent pattern of suppression was seen.

The *concertina* gene differs from its neighboring heterochromatic genes in being a strict maternal effect gene (SCHÜPBACH and WIESCHAUS 1989). We demonstrated in an earlier study (WAKIMOTO and HEARN 1990) that the wild-type cta^+ gene variegates in rearrangements that show very strong effects on *lt* variegation. This effect on cta^+ was detected in only 2 of the 17 rearrangements tested and was seen as decreased fertility of females heterozygous for either lt^{x18} or lt^{G10} and a *cta* mutant allele (WAKIMOTO and HEARN 1990). To determine if *cta* variegation could be enhanced by the *Su(var)* mutations, the fertility of lt^x/cta^{WU31} females with or without *Su(var)205* or *208* was compared. We did not detect an effect of *Su(var)205* on *cta* with either the lt^{x3} or lt^{x23} rearrangement (data not shown). However, *Su(var)208* enhances *cta* variegation in the lt^{x13} and lt^{x23} rearrangements (Table 5).

Taken together, the results described above allow us to conclude that the effects of *Su(var)205* and *208* on the 2L heterochromatic genes are general in nature. *Su(var)205* enhances the variegation of at least three (*40Fa*, *lt* and *40Ff*) and *Su(var)208* enhances the variegation of five of the 2Lh genes (*40Fa*, *40Fc*, *cta*, *lt* and *40Ff*).

The two remaining genes, *40Fe* and *40Fg* are the most proximally located essential genes in 2Lh (HILLIKER 1976). In a previous study, we failed to detect reduced expression of either gene in any of the lt^x rearrangements, even those known to move the genes to distal euchromatin (WAKIMOTO and HEARN 1990). In this study, we assayed for *40Fe* and *40Ff* variegation in the presence of the *Su(var)208* mutation. The results show that the viability of individuals that are heterozygous for *Su(var)208 EMS 56-24* or *Su(var)208 EMS 40-5* and any of the lt^x chromosomes tested was not significantly enhanced relative to $Su(var)^+$ controls (Table 4). In fact, the viability of *Su(var)208 EMS 56-24*/lt^{x3} females was significantly greater than *EMS 56-24*/lt^{x3} control females. Hence, we have no evidence to suggest that the *40Fe* and *40Fg* show PEV when displaced to distal euchromatin.

DISCUSSION

The results described above suggest that at least five of the genes located in the heterochromatin of chromosome *2* have different regulatory requirements than euchromatic genes, and that they require some of the *Su(var)* gene products for their proper expression. This conclusion is based on tests of 14 mutations that were isolated as strong suppressors of variegation of the *white* gene (SINCLAIR, MOTTUS and GRIGLIATTI 1983) for their effects on the variegation of genes in 2L heterochromatin. Six of the second chromosome *Su(var)* mutations, representing six genes, significantly enhanced the variegation of the *light* gene. Those with the strongest effects on *lt* were shown to enhance the variegation of several other genes in 2Lh. The *Su(var)208* mutation has been shown to enhance the variegation of the *40Fa*, *40Fc*, *cta*, *light* and *40Ff* genes and the *Su(var)205* mutation enhances the variegation of *40Fa*, *light* and *40Ff*.

Some of the mutations known to act as strong suppressors of the variegation of euchromatic genes had no detectable effect on the variegation of the *lt* gene. While several of the *Su(var3)* mutations had weak enhancing effects, none consistently enhanced *lt* variegation in both sexes or all three rearrangements tested. Only isolated cases of suppression of the variegation of the 2Lh genes were observed (see RESULTS). Since these cases of suppression of variegation occurred in only one sex or on only one rearrangement, these cases of apparent suppression may be due to random variations in our assays. While it is possible that some of the modifiers may suppress the variegation of both euchromatic and heterochromatic genes, the clearest cases involve reciprocal suppression of variegation of euchromatic genes and enhancement of variegation of heterochromatic genes.

The effect of *Su(var)* mutations on rearrangements with different heterochromatic breakpoints: Rearrangements that variegate for the 2Lh genes vary in their sensitivity to the *Su(var2)* mutations. Some of these differences may be due to differences in the position of the heterochromatic breakpoint. Rearrangements with breakpoints in the distalmost block of heterochromatin show the strongest variegation of the 2Lh genes (WAKIMOTO and HEARN 1990); in general, these rearrangements also appear to be more sensitive to the effects of the *Su(var2)* mutations than those that displace the bulk of 2Lh along with the variegating genes. For example, the *light* gene on lt^{x6} was more frequently affected by the *Su(var2)* mutations than the *light* gene on the lt^{x4}, lt^{x13} and lt^{x24} chromosomes. The effects of *Su(var)205* and *208* on the variegation of other 2Lh genes (the *40Fa* gene and the *40Fa*, *40Fc* and *cta* genes, respectively) were greater for the lt^{x23} chromosome than for rearrangements with more proximally located breakpoints. These observations are consistent with a model proposed by REUTER, WOLFF and FRIEDE (1985) to account for effect of *Su(var)* mutations on chromosomes generated as partial revertants of the $In(1)w^{m4}$ rearrangement. These authors suggest that the range of sensitivities of these partial revertants to two strong modifiers of position effect is due to the number of binding sites for heterochromatic proteins that remain on the revertant chromosomes. Similarly, sequences throughout 2L heterochromatin could act as binding sites for the *Su(var)* gene products. The lt^{x} rearrangements broken in h35 could move fewer of these binding sequences along with the *light* gene. When a $Su(var)^{+}$ gene product becomes limiting, for example due to *Su(var)* mutation, regions containing relatively fewer binding sites compete poorly for the protein and are most dramatically affected.

One of the *Su(var2)* mutations with a strong enhancing effect on variegation of the 2Lh genes shows properties consistent with this mechanism of action. The *Su(var)205* gene encodes a chromosomal protein HP1 (EISSENBERG *et al.* 1990) that localizes predominantly to heterochromatin (JAMES and ELGIN 1986). An antibody that recognizes this protein has been used to show that the displaced 2Lh in the lt^{x13} (JAMES *et al.* 1989) and lt^{x23} (M. G. HEARN, unpublished observations) rearrangements retains the ability to bind the protein in the salivary gland chromosomes. It is not clear, however, whether HP1 recognizes and binds to 2Lh sequences directly, or whether its association depends on other proteins in heterochromatin. As a structural component of the chromosome, HP1 may be required for the differential packaging of heterochromatin (JAMES and ELGIN 1986; SINCLAIR, MOTTUS and GRIGLIATTI 1983; LOCKE, KOTARSKI and TARTOF 1988; EISSENBERG 1989) that is necessary for activation of heterochromatic genes.

Alternatively, the wild-type products of the genes identified by *Su(var)* mutations that enhance variegation of the 2Lh genes may act as localization proteins. Rearrangements effective at inducing variegation of the 2Lh genes displace the genes to distal euchromatin. Many of the rearrangements, such as lt^{x2}, were complex involving three or more breakpoints. These would be expected to severely disrupt the ability of 2Lh to associate with other heterochromatic regions. If the 2Lh genes require proximity to large blocks of heterochromatin to acquire positive regulatory factors, a mutation that decreases the concentration of a protein required for localizing heterochromatin in a particular nuclear compartment might enhance variegation. Such localization proteins could bind to the nuclear matrix (GROSS and GARRARD 1987), facilitate interactions between homologs as has been proposed for the *zeste* protein (WU and GOLDBERG 1989; BICKEL and PIRROTTA 1990) or mediate interactions between different regions of hete ochromatin.

The products of the other *Su(var)* genes used in this study are unknown and certainly, the molecular mechanisms by which they act could be diverse. The *Su(var)* mutations that enhance *lt* variegation but suppress the variegation of euchromatic genes may be mutations in dosage sensitive genes that act indirectly on the variegating genes. For instance, they could control posttranslational modifications that might alter the ability of chromosomal proteins to bind to and maintain heterochromatin (MOTTUS, REEVES and GRIGLIATTI 1980; REUTER, DORN and HOFFMAN 1982;

EISSENBERG 1989). Alternatively, the *Su(var)* products may act directly on the 2Lh genes, for example as transcriptional regulators. Their reciprocal action as modifiers suggests that they would positively regulate heterochromatic genes, but negatively regulate euchromatic genes.

It has often been suggested that heterochromatic-euchromatic breakpoints cause position effects on euchromatic genes because of propagative changes in chromatin structure. Heterochromatin is commonly believed to be highly condensed and incompatible with gene function; in variegating rearrangements, heterochromatin would spread into adjacent sequences and render euchromatic genes inaccessible to inducing factors. We find it intriguing that a group of mutations that suppress variegation of euchromatic genes enhance variegation of heterochromatic genes. Similarly, two EMS-induced enhancers of *light* variegation have been shown to suppress the variegation of the *white* and *brown* genes (M. G. HEARN, unpublished observations). Our results suggest that the wild-type products of several of the *Su(var)* mutations are required for the normal function of genes in heterochromatin. Many of the *Su(var)* mutations exhibit pleiotrophic effects that include recessive lethality and female sterility (SINCLAIR, MOTTUS and GRIGLIATTI 1983; REUTER *et al.* 1986; SZABAD, REUTER and SCHRÖDER 1988). It will be interesting to determine if the effects attributed to the *Su(var)* mutations are due to the reduced expression of essential genes located in heterochromatin.

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