CHARACTERIZATION OF A CLUSTER OF DOMINANT SUPPRESSORS OF POSITION EFFECT VARIEGATION INCLUDING EFFECTS ON HETEROCHROMATIC VARIEGATING REARRANGEMENTS IN *DROSOPHILA MELANOGASTER*

By

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B.Sc., Portland State University, 1985

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS OF THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Zoology)

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

April 1989

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ABSTRACT

The mosiac, cell-autonomous expression of genes resulting from chromosomal rearrangement and relocation next to broken heterochromatin is termed position effect variegation (PEV). Since the gene is inactivated due to chromatin changes, this system allows the genetic study of chromatin structure and function using mutations which rescue the mosaic phenotype. These mutations called suppressors of variegation, Su(var)s, must influence chromatin structure. The genetic characterization of several groups of Su(var)s has been undertaken in this study using *Drosophila melanogaster*.

Variegation of the *light* gene, located in heterochromatin, is enhanced by several Su(var) mutations on chromosome two. This opposite effect suggests that products of these Su(var)s are essential for functioning heterochromatin and deleterious for euchromatic environments. Other Su(var)s have slight or no effects on the same variegating rearrangements, demonstrating functional differences among the Su(var)s tested.

A group of Su(var)s located within 4 map units near the centromere of chromosome three was characterized using deficiency mapping, new compound autosome formation and *inter se* complementation based on newly established

homozygous phenotypes. Two Su(var)s mapped to 87B on 3R, while one Su(var) maps to 3L according to compound mapping. *Inter se* complementation, in combination with mapping data, suggests that four seperate loci make up this group of Su(var)s.

Eight of nine Su(var)s are extremely sensitive to heterochromatic deletions as shown by their responses to loss of 2R heterochromatin, as well as the Y chromosome. In contrast, Su(var)A130 is insensitive to both forms of heterochromatic deficiencies. Su(var)s show complicated reactions to maternal verses paternal source effects. Six of nine Su(var)s show a female-specific temperature sensitive maternal effect. Some maternal and paternal effects are observed at 22 C. Su(var)A57 is maternal semi-lethal and suppressed at 29 C. This characterization has better defined these mutants, making them ammenable to molecular study.

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ACKNOWLEDGEMENTS

I thank my supervisor, Tom Grigliatti for allowing me independence of research, and for always taking time to help with advice and encouragement. Thanks also to Don Sinclair for his support and input throughout this study and Hugh Brock for spending time to read the rough drafts.

Especially, I would like to thank my husband, Michael and my fellow fly pushers, Jo-Ann Brock and Joanie McKeon for invaluble discussion, advise, encouragment and support. Finally, thanks to all the zoology geneticists who made graduate school enjoyable.

GENERAL INTRODUCTION

Position effect variegation (PEV) results when a gene is moved via chromosome rearrangement from its usual location to a position next to a newly formed euchromatic-heterochromatic boundary. The rearranged gene is inactivated in some cells, but remains active in others. This on/off decision is made early in development and is clonally propogated, resulting in mosaic expression of the gene. Both euchromatic and heterochromatic genes are subject to this phenomena, but the majority of work has been done with euchromatic variegating genes. PEV as defined above was first described by Sturtevant (1925). Since then, most work has been done in *Drosophila melanogaster* where virtually every gene tested is subject to PEV (for reviews, see Lewis 1950; Baker 1968; Spofford 1976).

Although much progress has been made since 1925, the molecular mechanism of PEV is still unknown. Judd (1955) has provided evidence against mutation resulting in inactivated genes, and Henikoff (1979) has ruled out somatic gene loss as a cause of mosaic expression. This suggests that changes in chromatin structure are the cause of variegating position effects.

Zuckerkandl (1974) proposed that heterochromatic elements spread beyond the disrrupted heterochromatic boundary, causing transcriptional inactivation of

neighbouring euchromatic loci through altered chromatin structure. There is evidence to support such a model. First, PEV shows a spreading or polar affect. Genes are inactivated in order of their proximity to the heterochromatic breakpoint. Genes farther away will not variegate unless other genes between are also variegating (Demerec and Slizynska 1937; Schultz 1941; for an exception see Chovnick and Clark 1986). This supports the idea that condensing molecules spread outward from heterochromatin and inactivate euchromatic genes. Second, Hartman-Goldstein (1967) and more recently Zhimulev *et al.* (1986) have correlated heterochromatin-like morphology of salivary gland chromosomes with variegating phenotypes.

The initiation of this spreading has been investigated by Tartof *et al.* (1984). By cloning the heterochromatic junctions of three standard variegating rearrangements (*wm4, wm51b, wmMc*) they have shown that the euchromatic-heterchromatic boundary is flanked by mobile element-like sequences. These sequences alone are not capable of inducing chromatin condensation. This suggests that heterochromatization is initiated far away from the breakpoint, within the heterochromatin. However, Reuter *et al.* (1985) have found complete phenotypic revertants which retain sensitivity to dominant enhancer mutations of PEV. They conclude that the euchromatic-heterochromatic junction flanked by one or more of these sequences is sufficient to cause variegation.

PEV and its associated chromatin changes can be investigated due to the existance of several potent modifiers of the PEV phenotype. All variegating phenotypes are suppressed by high developmental temperature, so that the variegating gene is expressed in a greater number of cells. Low developmental temperature enhances variegation, causing the gene to be inactivated in more cells (Gowan and Gay 1933). Another standard modifier of PEV is heterochromatin content of the cell. Variegation is enhanced by loss of heterochromatin such as the Y-chromosome (Gowan and Gay 1934) and the centric heterochromatin deletion Df(2R)*M-S210* (Morgan et al. 1941). Conversly, an extra Y-chromosome suppresses PEV. These observations suggest that the presence of heterochromatin in a cell acts as a sink for heterochromatic elements. Extra heterochromatin attracts more of these elements away from the facultative spreading heterochromatin, thus leaving the rearranged gene in a euchromatic environment, resulting in a less extreme mutant phenotype. Loss of heterochromatin frees these elements and allows formation of heterochromatin at the breakpoint, causing a more severe mutant phenotype. This sink effect may be due to heterochromatin content, or to specific regions (binding sites) in heterochromatin. Brock (1986) reports that different regions of the Y-chromosome have an enhancing effect on variegation which is not correlated with size.

More recently discovered modifiers of PEV include histone gene multiplicity, and butyrate, an inhibitor of histone de-acetylases. Moore *et al.* (1979; 1983) demonstrated that variegating phenotypes are suppressed by a histone deficiency. This suggests that a decrease of histones available to the chromatin limits transcriptional inactivation due to heterchromatic packaging, thus allowing a more wild type phenotype. In addition, Mottus *et al.* (1980) have found that sodium butyrate can suppress variegation, probably since histone de-acetylases are inhibited, resulting in acetylated histones. These findings support the heterochromatization mechanism for PEV gene inactivation.

Genetic modifiers of PEV are perhaps the most useful investigative tools. Spofford (1967) identified the first dominant suppressor of PEV (Su(var)) mutation. Since then, Reuter and Wolff (1981) and Sinclair *et al.* (1983) have independently isolated over 150 X-ray or EMS induced dominant Su(var) mutations. The Su(var)s were selected for their effects on the *white* gene variegation rearrangement, *wm4*, but show general effects, suppressing both *brown* and *scute* variegation (Reuter and Wolff 1981) and *brown* and *Stubble* variegation (Sinclair *et al.* 1983).

Genetic mapping revealed that many of the 50 Su(var)s of Sinclair *et al.* (1983) fall into discrete clusters defined as mutants originally mapping within a three centimorgan distance. Reuter and Wolff (1981) have

suggested this label is unnecessary, but for the purposes of discussion these Su(var)s will be referred to as the 2L clustered and nonclustered Su(var)s, and the 3R proximal, middle and distal clusters.

Characterization of Su(var) mutations is the subject of this thesis. First, interactions between Su(var)s and heterochromatic variegators, heterochromatic loci that variegate as a consequence of relocation near to euchromatin, are investigated to determine the relationship between heterochromatin and gene inactivation. Second, a 3R proximal cluster is characterized with regard to standard modifiers of PEV and genetic relationship of the Su(var)s in this cluster, to provide a necessary knowledge base for possible molecular experimentation.

CHAPTER 1 - The effect of Su(var) mutations on *light* gene variegation.

INTRODUCTION

Questions about chromatin packaging are fundamental to understanding gene expression. Position effect variegation (PEV) causes heterochromatinization of the DNA that is also correlated with gene inactivation (see general introduction). Thus, PEV provides a model system for studying heterochromatin and its role in gene regulation. Both euchromatic and heterochromatic loci are subject to PEV. Using euchromatic and heterochromatic loci which variegate, differences and similarities in the mechanisms of gene inactivation can be studied.

This study utilizes a heterochromatic gene, *light* (*lt*) which exhibits a mosaic phenotype when moved away from centric heterochromatin and relocated next to distal euchromatin (Schultz and Dobzhansky 1934). The variegation of *lt* appears in many ways to be reciprocal to standard euchromatic PEV (euPEV), such as variegation of the *white* gene. *Light* variegation, then, is one example of heterochromatic PEV (hPEV). (See Spofford 1976 for review of PEV and Hannah 1951;Hilliker *et al.* 1980, for reviews of heterochromatin).

Few examples of hPEV are documented, probably since so few genes have been mapped to heterochromatin. Three heterochromatic genes are known to variegate: *peach* in *D. virilus*, Baker (1953); *cubitus interruptus (ci)* in *D. melanogaster*, Khvostov (1939); and *light*, also in *D.*

melanogaster, Hessler (1958). One requirement for hPEV has been observed. In order for a heterochromatic gene to variegate, the position of the euchromatic breakpoint must be in the distal two-thirds of the chromosome (or in other heterochromatin which is then disrupted). Baker (1968) suggests that a repair mechanism spreads from the undisturbed heterochromatin. A gene that is relocated to distal euchromatin cannot be reached by this competent heterochromatin and, therefore, repair cannot take place.

While *light* variegation follows this requirement for hPEV, it does demonstrate some irregularties. *Light* variegating rearrangements (lt^{XV}s) show various phenotypes (Hessler 1958). Some rearrangements result in a pale-mottled eye, having many *light* ommatidia and few wild-type cells. Others show a dark-mottled eye, which appears as a wild-type eye interspersed with darker ommatidia. Some rearrangements give phenotypes intermediate between these extremes. Hessler (1958) found no correlation between cytological breakpoints and any one phenotype.

All *lt* phenotypes respond to standard modifiers of PEV, such as temperature and the presence or absence of heterochromatin (Baker 1968). *Light* variegation is suppressed by elevated temperature as is euchromatic variegation. However, addition of a Y chromosome (normally a suppressor of PEV) acts as an enhancer of *lt* variegation. This observation sets *lt* apart from other heterochromatic

variegators (ie; *peach* and *ci*) and suggests some aspects of *lt* variegation are reciprocal to euPEV.

If the mechanism of *lt* variegation is reciprocal to euchromatic variegation, its movement into distal euchromatin is likely associated with chromatin changes which are detrimental to proper expression of the gene. The response of *lt* variegation to the heterochromatic Y chromosome implies sensitivity to the amount of heterochromatin in the cell which is opposite to that of euchromatic variegation. Dominant modifiers of PEV can be employed to investigate the response of heterochromatic variegators to alterations in chromatin.

Dominant suppressors of PEV (Su(var)s) are able to completely reverse the inactivation of genes which results from abnormal proximity to heterochromatin. It is likely that these Su(var) genes encode products involved with heterochromatin (see main introduction). If *light* variegation is mechanically reciprocal, one can expect Su(var)s to act as enhancers of *light* variegation, due to further stress on heterochromatin in the cell. This study examines the effect of several dominant suppressors of euchromatic PEV on various *light* variegating rearrangements.

MATERIALS AND METHODS

STOCKS: Mutations used in this study not listed in Lindsley and Grell (1968) are listed in Tables 1 and 2. All suppressors of variegation, Su(vars), were isolated as described by Sinclair *et al.*, (1983). *Light* variegating rearrangements were isolated and characterized by Barbara Wakimoto (personal communications).

Since the third chromosome Su(var)s do not carry *lt* mutations, it was necessary to construct two stocks, diagrammed in Figure 1. These stocks provide a *lt* background so that *lt* variegation can be detected in the presence of these Su(var)s.

CULTURE CONDITIONS: Flies were raised at 22 C (unless otherwise stated) on standard cornmeal-sucrose *Drosophila* medium. Tegosept was added as a mould inhibitor.

EFFECT OF SU(VAR)S ON *LIGHT* VARIEGATION: To test the effect of second chromosome suppressors (Su(var)II)s on *lt* variegation, the following cross was performed. Males of the genotype *wm4;*Su(var), *b lt rl/CyO* were crossed to +/+; *ltxv/Gla* or *ltxv/ltxv* virgins. F1 heterozygotes were collected 0-3 days post-eclosion, aged 5 days, and pigment analysis was performed (see below).

TABLE 1: SUPPRESSOR OF POSITION EFFECT VARIEGATION USED IN THIS STUDY.

Mutations	Alternate Designation	Map position (± 95% confidence levels)
2L CLUSTERED		
Su(var)214	*Su(var)A24*	34.9 ± 1.8
Su(var)210	*Su(var)C157*	34.8 ± 1.8
Su(var)207	*Su(var)H69*	32.0 ± 1.4
Su(var)216	*Su(var)M59*	34.2 ± 1.6
2L NONCLUSTERED		
Su(var)208	*Su(var)T44*	5.7 ± 1.3
Su(var)206	*Su(var)A151*	51.3 ± 0.9
Su(var)205	*Su(var)M43*	30.8 ± 1.6
Su(var)201	*Su(var)B89*	45.9 ± 3.5
3R PROXIMAL		
Su(var)310	*Su(var)A63*	54.4 ± 0.7
Su(var)316	*Su(var)A48*	47.4 ± 0.8
Su(var)308	*Su(var)A57*	49.2 ± 1.2
Su(var)307	*Su(var)B94*	47.4 ± 0.9
Su(var)304	*Su(var)B143*	46.4 ± 1.1
Su(var)321	*Su(var)C119*	47.6 ± 1.3
Su(var)319	*Su(var)B76*	48.6 ± 1.3
3L ARM		
Su(var)323	*Su(var)A130*	3L see appendix
Su(var)303	*Su(var)A160*	3L

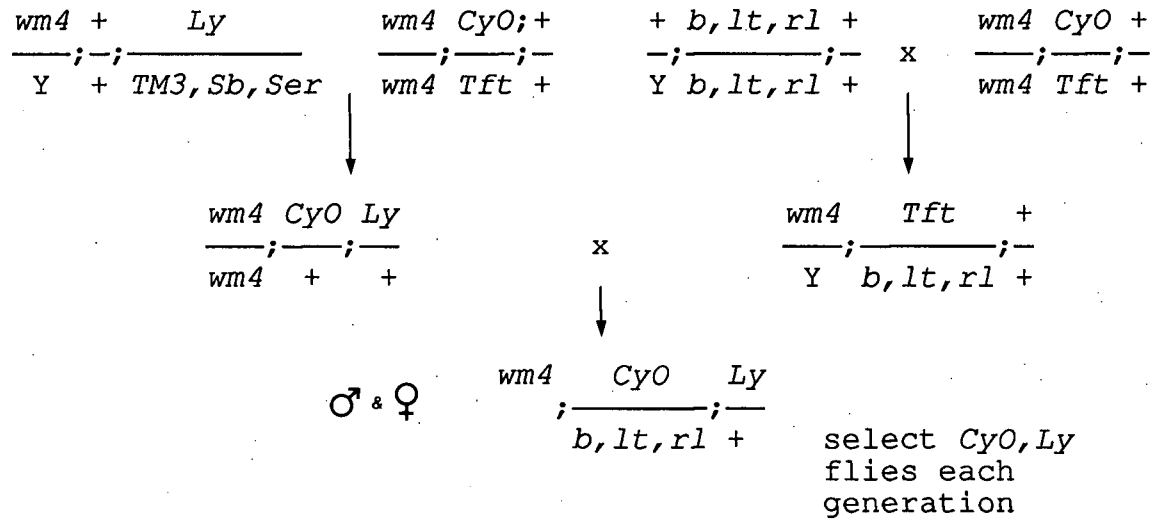
TABLE 2: DESCRIPTION OF *LIGHT* VARIEGATING REARRANGEMENTS

LIGHT REARRANGEMENT (abrieviation)	PHENOTYPE	CYTOLOGY
In(2L)*ltx18* (*ltx*18)	pale-mottled	T(2;3)40?, 94D + T(2;3)32C, 64
T(2;3)*ltx6* (*ltx6*)	moderately-mottled	insertional translocation of 63-74 to 36h*
T(2;3)*ltx*2 (*ltx*2)	moderately-mottled	In(2L)25D5, 40A + In(2LR)40A, 53A1,2
T(2;3)*lt13* (*lt13*)	dark-mottled homozygous viable	T(2;3)37h; 97D2
T(2;3)*ltx*24 (*ltx*24)	dark-mottled,	T(2;3)37,38h; 61D3-61F
T(2;3)*ltx4* (*ltx4*)	dark-mottled	T(2;3)36,37h;97D1

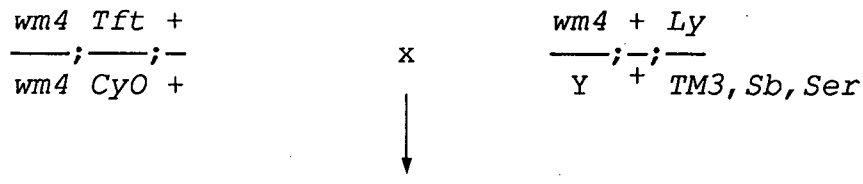
*h indicates heterochromatic cytology units in 2L heterochromatin (unpublished map).

FIGURE 1: CONSTRUCTION OF STOCKS FOR TESTING THIRD CHROMOSOME SU(VAR)S WITH *LIGHT* VARIEGATING REARRANGEMENTS

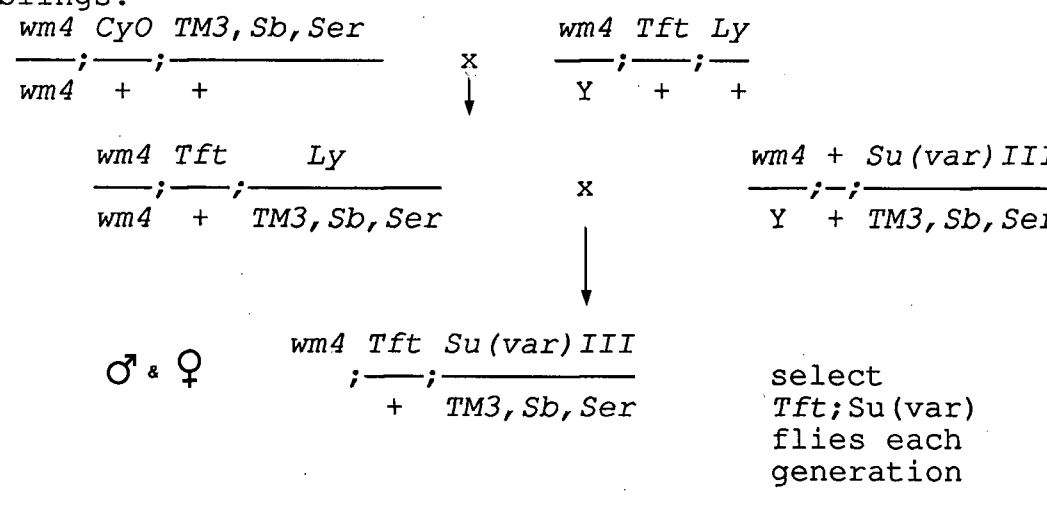
To provide *b,lt,rl* background:



To mark the second chromosome of *Su(var)IIIs*:



F1 siblings:

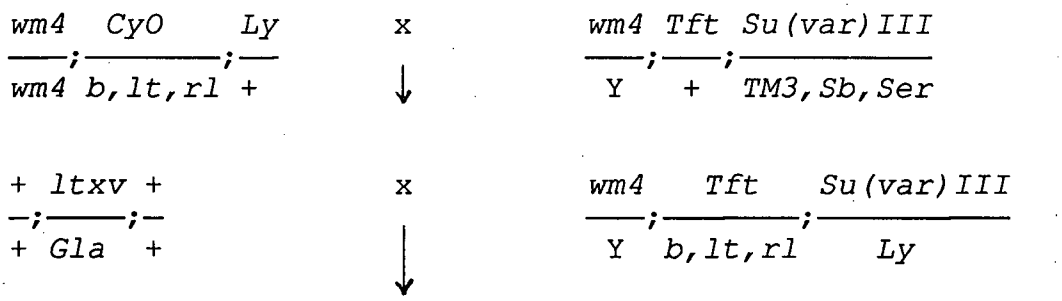


To test the effect of Su(var)IIIs on *lt* variegation (chromosome 2) a second chromosome containing a *lt* mutant allele was required in the Su(var)III strains. This was accomplished with the protocol shown in Figure 2. *ltxv;*Su(var) F1 progeny were collected 0-3 days post-eclosion, aged 5 days and subjected to pigment analysis (see below).

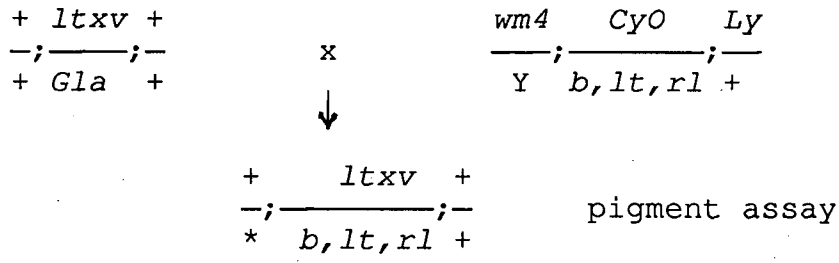
FLUOROMETRIC ASSAY OF EYE PIGMENT: To measure the effect of the Su(var)s on *lt* variegation, eye pigments were extracted and analyzed as follows. Adults aged 5-8 days post-eclosion were frozen in glass tubes at -70 C. Flies were decapitated immediately after removal from -70 C by firmly banging the tubes. A total of 50 heads for each genotype were placed in 1.5 ml Ependorf tubes, 5 heads per tube, males and females separated at collection. Pigment was extracted by sonication of heads in 30 ul of 0.25M 2-mercaptoethanol in 1% aqueous NH_4OH. After brief centrifugation, samples were placed in the dark for approximately 1 hour at room temperature. The head fragments were precipitated by centrifugation at 12,000 G for 2 minutes. 5 ul of the supernatant from each sample was pippetted onto Whatman #3 filter paper fixed to a microscope slide so that each slide contained five 5 ul aliquots of one genotype. The amount of pigment was quantified by flourescence at >500 um (in the linear range of the instrument) using a Zeiss

FIGURE 2: CROSSES TO TEST EFFECTS OF *SU(VAR)III* MUTANTS ON *LIGHT* VARIEGATING REARRANGEMENTS

Experimental:



Control:



*Chromosome is either an X carrying *wm4* or a Y.

microfluoremeter. Pigment levels are expressed as a percentage of wild type (Oregon-R) pigment levels.

STATISTICS: Fluorescence values expressed as percentages of Oregon-R control pigment levels, which form a binomial distribution, were transformed to their arcsine values to approximate a normal distribution. Su(var)II values were compared with control values by ANOVA followed by Dunnett's multiple range test (Zar 1984). Differences between Su(var)III values and their internal control values were determined by unpaired t-tests. The statistical limit of significance was taken as $P<0.05$.

RESULTS

To measure effects of Su(var)s on *lt* variegation, it is first necessary to analyze strengths of each *lt* variegating strain. All control crosses for Su(var)II experiments were performed three separate times and the results are listed in Table 3. Notice that *lt/lt* pigment levels are very consistent, ranging from 30.3±1.2 to 34.2±1.0 percent of Oregon-R pigment. As expected, basal levels of *lt* variegating strains are more variable; in fact, the variability is so great that pigment values are often significantly different between trials. Even so, it is possible to group these variegators into 3 categories of variegating strength. First, *ltx18* is a very strong variegator (pale mottled) often showing *lt/lt* pigment levels, indicating that the *light+* gene is inactivated in approximately 100% of the ommatidia. Suppression of variegation by Su(var)s may be observed in this strain since increases in pigment could be easily detected. *ltx6* and *ltx2* are moderate variegators, producing between 40 and 65% of Oregon-R pigment levels. Suppression or enhancement may be detected in these strains since their pigment levels can range upward or downward from basal variegating levels. Finally, *ltx13*, *ltx24* and *ltx4* are very weak variegators (dark mottled) usually producing pigment between 70 and 100% of Oregon-R pigment levels. Therefore, suppression by

TABLE 3: BASAL *LIGHT* VARIEGATING CONTROL PIGMENT LEVELS FROM THREE SEPERATE TRIALS

GENOTYPE	TRIAL 1 (mean ± 2 S.E.)	TRIAL 2 (mean ± 2 S.E.)	TRIAL 3 (mean ± 2 S.E.)
lt/lt			
male	32.5 ± 1.5	30.3 ± 1.2	33.0 ± 2.0
female	31.2 ± 2.3	33.5 ± 1.2	*34.2 ± 1.0
ltx18			
male	26.9 ± 1.4	26.7 ± 2.5	31.6 ± 1.9
female	*31.0 ± 3.6	*24.6 ± 3.9	*40.9 ± 6.0
ltx6			
male	64.1 ± 8.2	53.3 ± 4.6	54.1 ± 8.1
female	*50.1 ± 4.9	42.1 ± 3.4	41.8 ± 3.5
ltx2			
male	54.6 ±10.9	46.2 ± 9.5	45.2 ± 3.7
female	*57.0 ± 5.5	*47.0 ± 7.3	*38.5 ± 2.0
ltx13			
male	84.4 ± 4.7	*91.5 ± 7.0	80.0 ± 4.7
female	66.1 ± 5.2	*105.0 ± 7.1	70.7 ± 0.9
ltx24			
male	*102.5 ± 2.5	77.5 ±10.5	81.8 ± 3.3
female	82.3 ± 5.7	89.8 ± 4.0	*76.0 ± 2.7
ltx4			
male	118.7 ± 2.7	93.4 ± 9.6	77.1 ± 6.3
female	88.2 ± 2.6	91.7 ± 4.6	*70.6 ± 2.9

*significantly different from other control values within that genotype and sex.

Su(var)s would probably be undetectable in these strains since higher pigment levels may be indistinguishable from basal *ltxv* levels. Enhancement, or reduced pigment could be readily detected.

Su(var)s used in these experiments can also be grouped into categories: clustered and nonclustered chromosome two suppressors and third chromosome suppressors (see Table 1) Results of the effects of both groups of Su(var)II suppressors on *lt* variegation are shown in Figures 3a-f (actual pigment values are listed in the appendix). Two trials were completed for most combinations of *ltxv*s with Su(var)II mutants. Trial I was assayed in one sitting, while trial II was split into 2 assays, each having individual control *ltxv* levels measured. Results of the effects of Su(var)III mutants were more difficult to obtain because of stock viability problems. Therefore, one strain from each category of *lt* variegation strength was chosen for analysis of the third chromosome Su(var)s. Pigment data for these crosses are shown in Table 4. Each *ltxv;*Su(var) pigment level is shown with an internal control value taken from *ltxv;Ly* siblings. Differences between these values indicate effects of the Su(var) mutation versus a non-suppressor chromosome marked with *Lyre*.

Pigment levels are measured for males and females independently, to account for any sexually dimorphic properties common among Su(var) mutants. Although many cases show that males and females react differently to a

FIGURE 3a-f: Effects of Su(var)II mutations on *light* variegation. Shaded areas indicate basal *light* variegating pigment levels. Open bars indicate pigment levels of *Su(var)II/ltxv* heterozygotes. Values are mean $\pm$ 2 S.E.

FIGURE 3a: Pigment levels of Su(var)II/Itx18 heterozygotes.

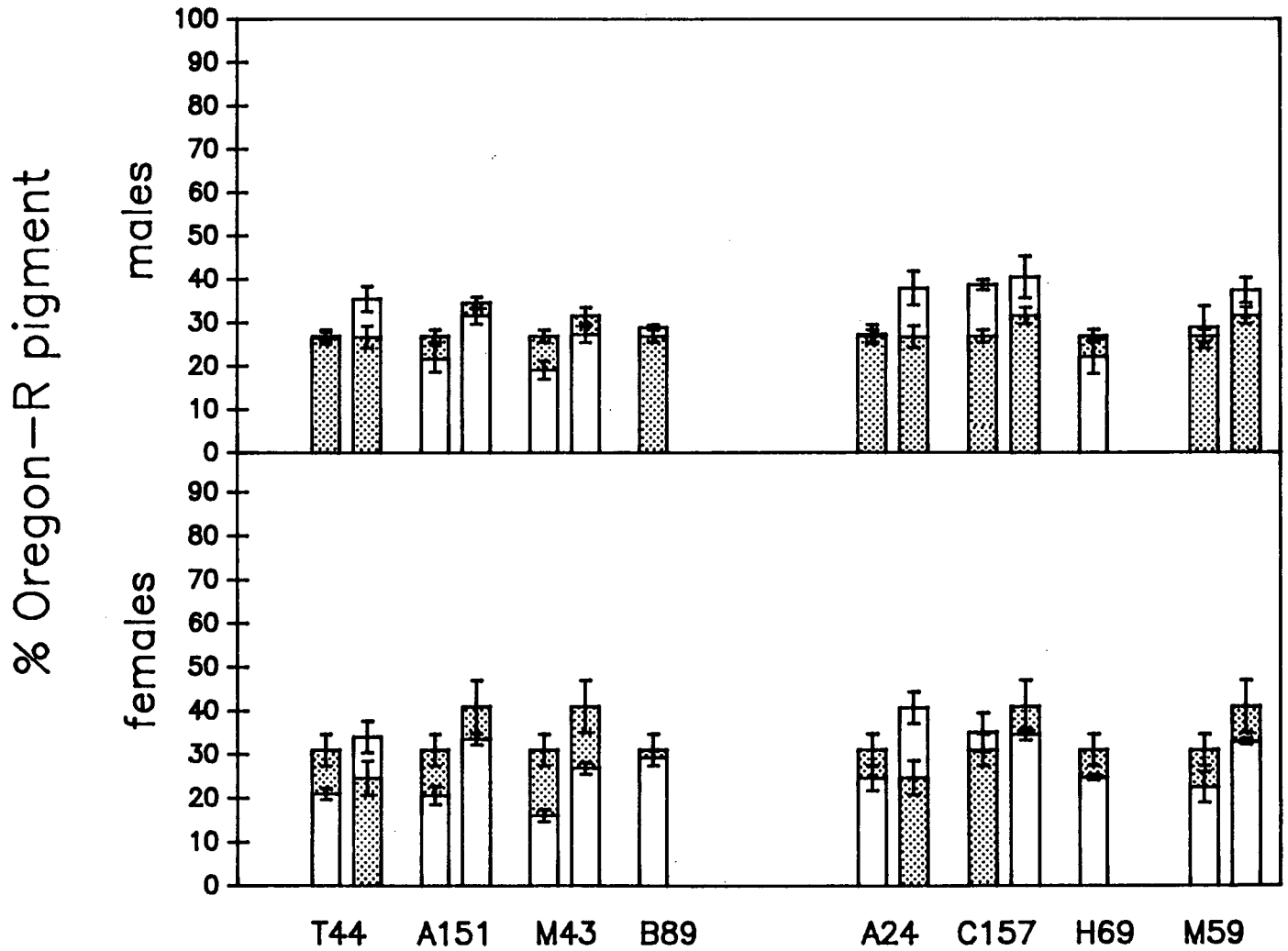


FIGURE 3b: Pigment levels of Su(var)II/ltx6 heterozygotes.

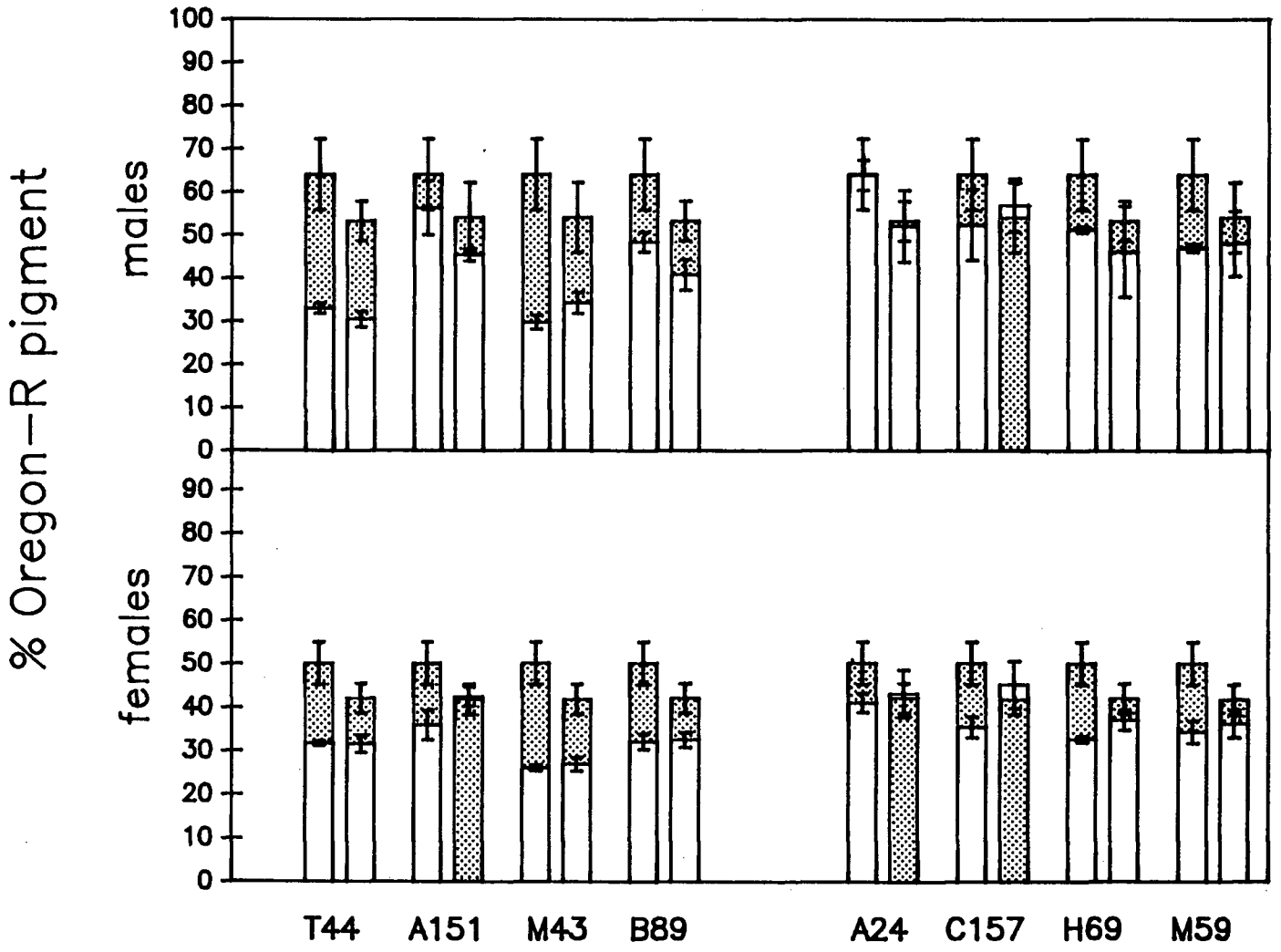


FIGURE 3c: Pigment levels of su(var)II/ltx2 heterozygotes.

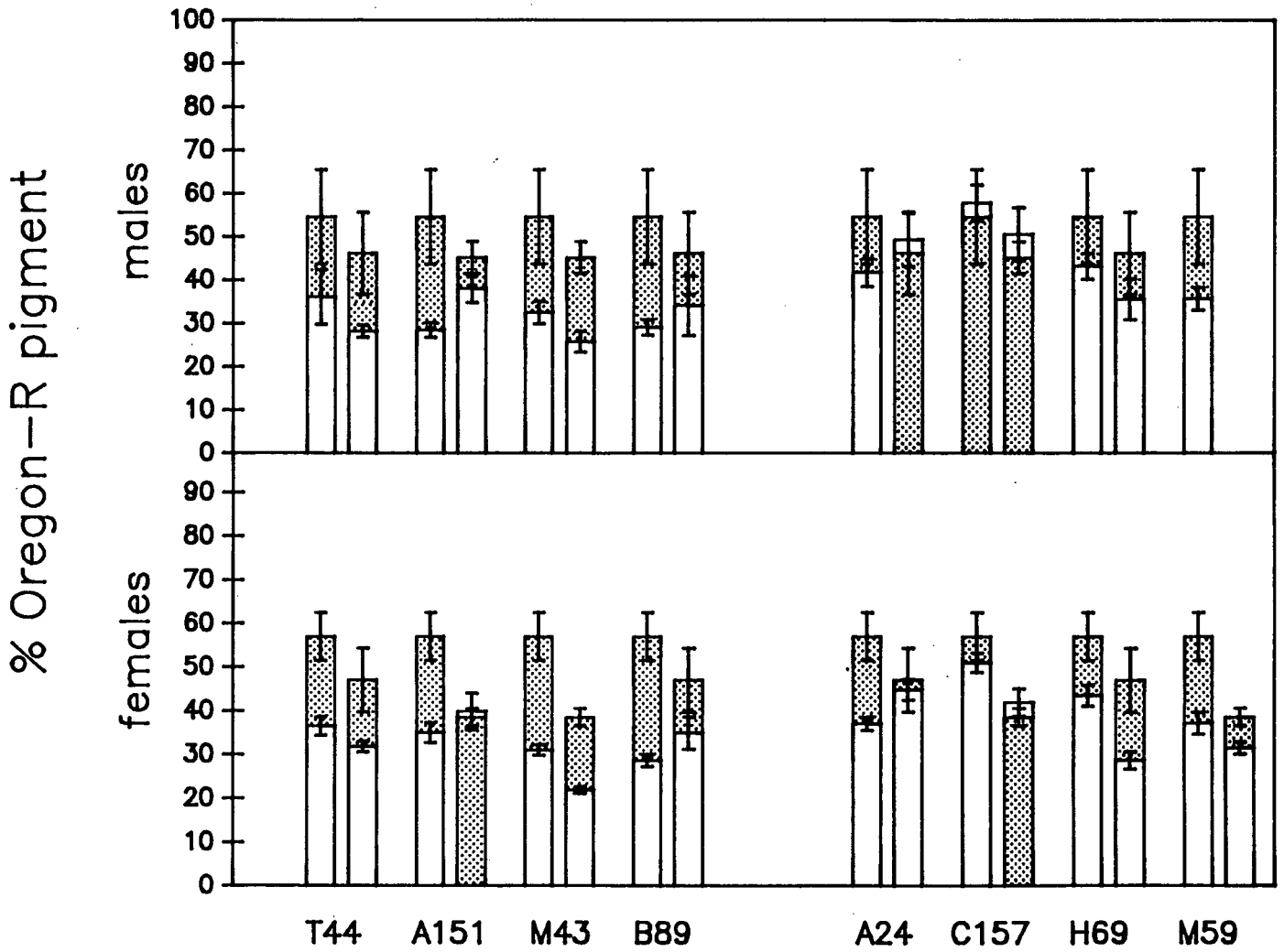


FIGURE 3d: Pigment levels of Su(var)II/Itx13 heterozygotes.

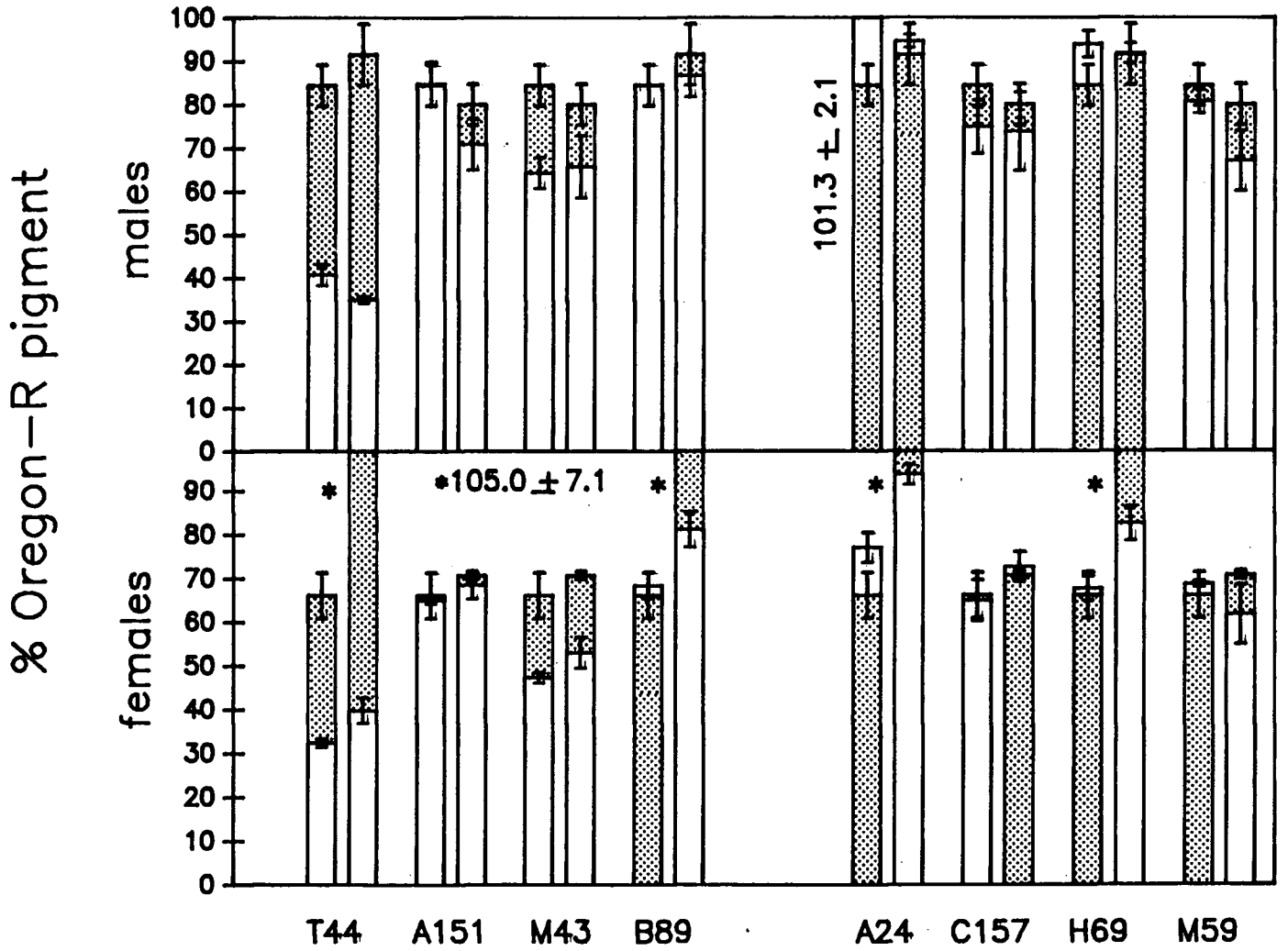


FIGURE 3e: Pigment levels of Su(var)II/ltx24 heterozygotes.

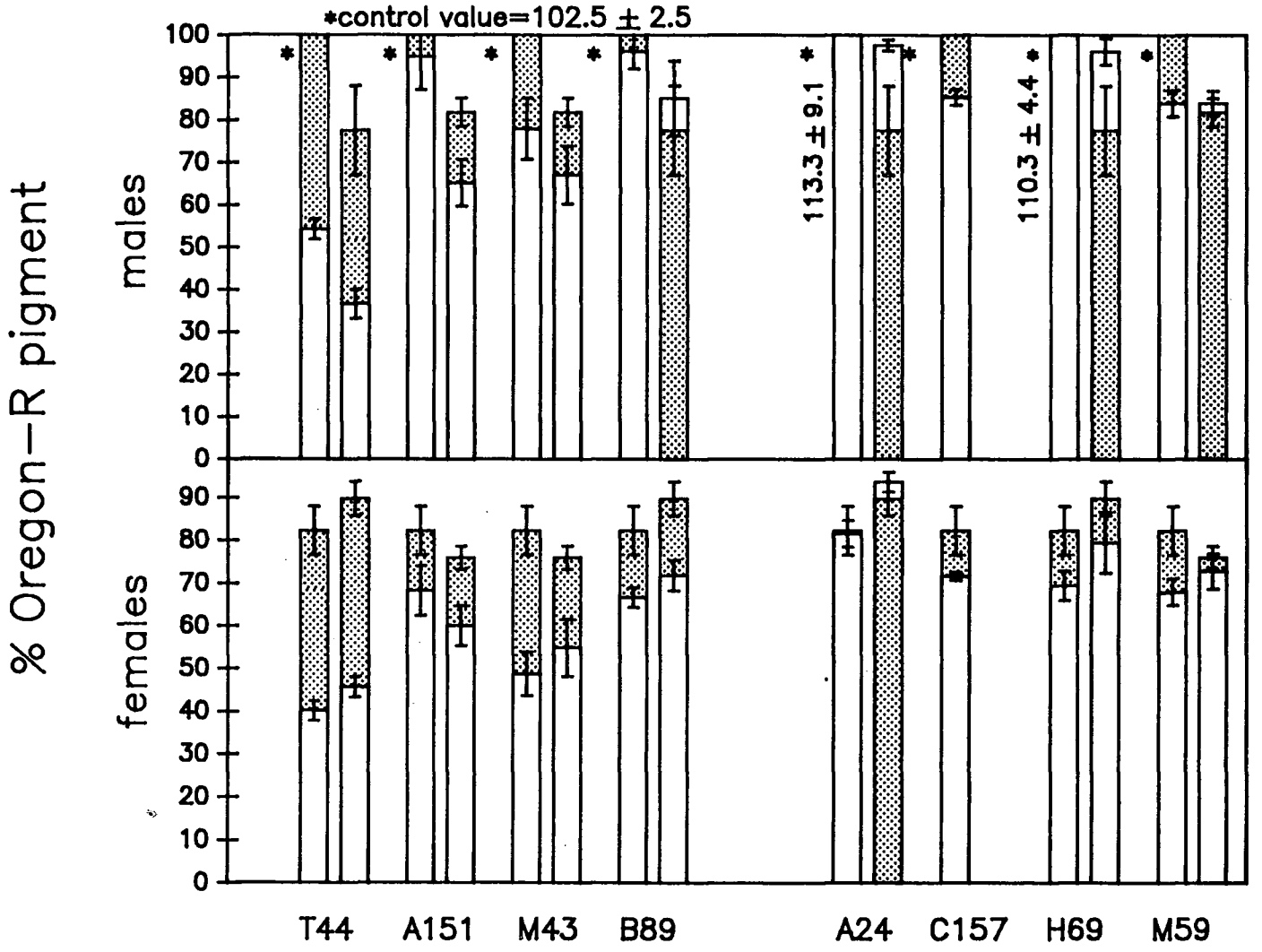


FIGURE 3f: Pigment levels of Su(var)II/ltx4 heterozygotes.

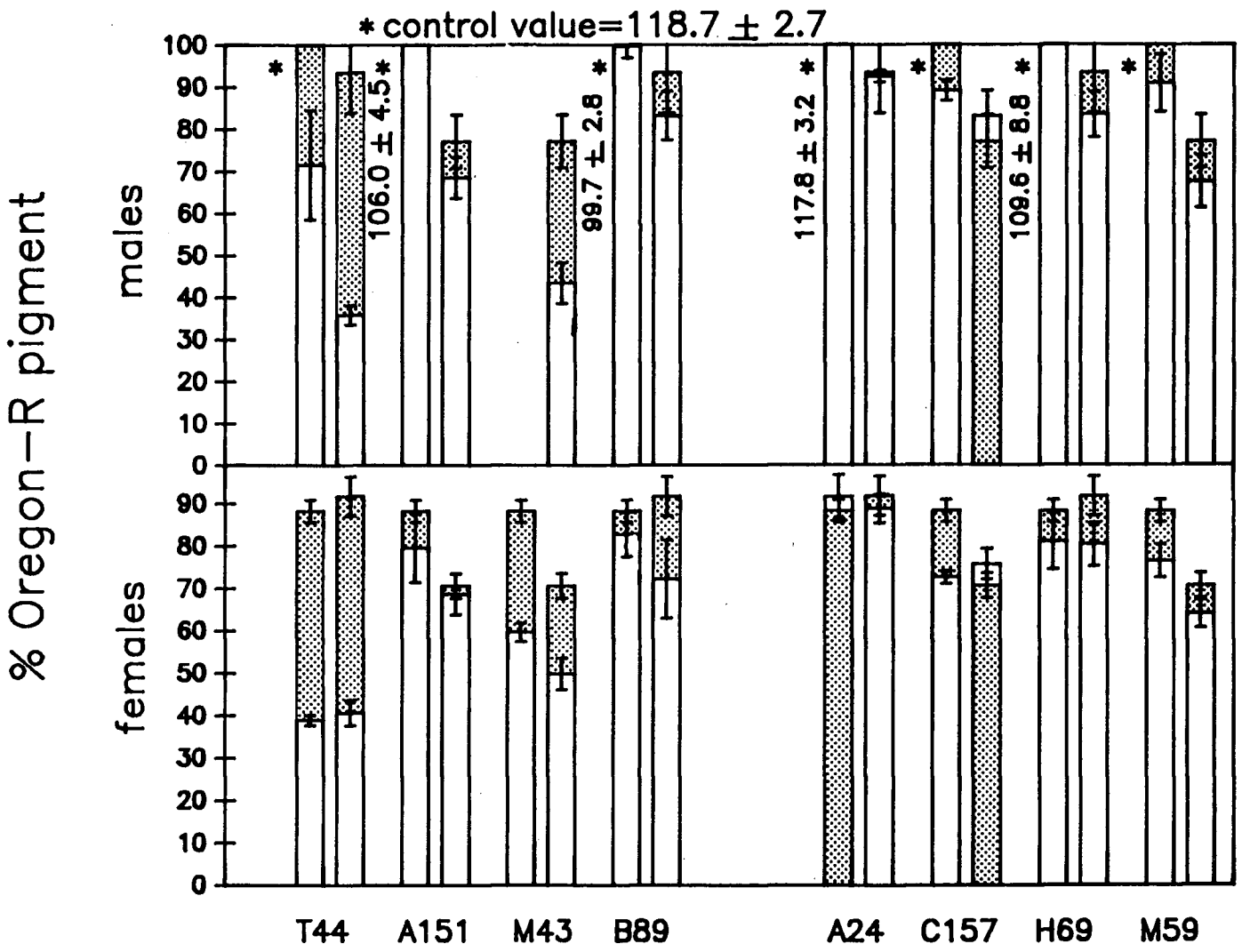


TABLE 4: EFFECTS OF SU(VAR)III MUTATIONS ON THREE *LIGHT* VARIEGATING STRAINS

GENOTYPE	MALE (mean ± 2 S.E.)		t-value	FEMALE (mean ± 2 S.E.)		t-value
ltx13;+	86.4	10.8		71.4	4.0	
ltx13;B94	70.5	4.4		63.0	2.6	
			t=0.98			t=2.14
control	73.9	6.2		57.9	4.2	
ltx13;B76	76.9	6.1		70.2	3.0	
			t=0.82			t=4.31*
control	80.3	5.5		57.2	6.1	
ltx13;C119	73.7	5.4		70.5	2.5	
			t=1.90			t=0.36
control	82.8	2.8		71.3	3.9	
ltx13;A63	83.4	5.8		63.9	4.7	
			t=0.20			t=1.17
control	82.5	6.6		59.7	5.6	
ltx13;A48	60.8	8.5		59.3	4.8	
			t=2.96*			t=0.86
control	68.0	1.1		61.6	2.7	
ltx13;A57	74.8	5.0		62.1	1.8	
			t=0.62			t=2.28*
control	76.5	3.4		58.7	2.4	

t(8)=2.31 t(7)=2.37 *=significant difference

control=internal control, ie;*ltxv;Ly*

TABLE 4: continued

GENOTYPE	MALE (mean ± 2 S.E.)		t-value	FEMALE (mean ± 2 S.E.)		t-value
ltx6;+	45.8	2.0		35.2	8.2	
ltx6;B94	36.3	7.9	t=1.92	31.4	1.3	t=1.4
control	43.6	3.8		30.3	0.7	
ltx6;B76	36.2	3.7	t=2.67*	35.2	1.0	t=2.27
control	41.3	2.3		32.3	2.8	
ltx6;C119	36.6	2.9	t=2.42*	33.5	2.0	t=2.09
control	43.6	5.5		39.7	8.6	
ltx6;A63	49.8	6.0	t=1.01	34.6	6.0	t=2.06
control	47.4	1.7		30.0	1.3	
ltx6;A48	39.9	3.8	t=1.14	24.6	0.8	t=2.64*
control	43.1	4.4		21.8	2.9	
ltx6;A57	32.6	2.9	t=2.33*	27.8	1.8	t=2.41*
control	38.2	3.9		24.2	2.5	

TABLE 4: continued

GENOTYPE	MALE (mean ± 2 S.E.)		t-value	FEMALE (mean ± 2 S.E.)		t-value
ltx2;+	33.1	3.4		27.9	0.7	
ltx2;B94	29.3	6.1		24.4	1.4	
			t=0.84			t=0.55
control	32.4	5.0		25.0	1.7	
ltx2;B76	23.9	2.3		30.1	1.9	
			t=2.06			t=2.86*
control	27.7	2.7		36.7	5.3	
ltx2;C119	35.7	2.0		30.3	3.9	
			t=0.86			t=0.45
control	37.3	3.4		31.6	4.3	
ltx2;A63	33.0	2.4		33.1	5.1	
			t=0.51			t=1.32
control	35.2	7.7		29.9	2.4	
ltx2;A48	28.7	1.3		30.8	3.0	
			t=5.42*			t=0.47
control	36.3	3.1		29.8	2.9	
ltx2;A57	33.0	4.8		30.1	3.4	
			t=2.68*			t=4.08*
control	36.6	4.8		25.3	0.8	

particular Su(var), these effects are not consistent between trials for Su(var)IIs and differences are small (t-values are low) for Su(var)III mutations tested.

The variability associated with PEV, as observed in basal *lt* variegating pigment levels, complicates analysis of these data. Several factors may contribute to this variation, including the inherent variability of these strains, the pigment assay system and pipetting error. Linearity tests (see appendix) have shown that higher amounts of pigment approach the end of the linear range of the instrument. Pipetting errors also potentially contribute to the overall error since very small amounts of pigment are used.

In this study, standard statistical techniques were applied to determine significant changes from basal *lt* variegating levels due to Su(var) action. However, given the inter-trial variability of control basal *lt* variegating levels, pigment differences between Su(var)II/*ltxv* flies and control *ltxv* levels were considered biologically significant if two criteria were met: 1) statistically significant differences were consistent between trials and 2) the direction of change (i.e. enhancement or suppression) was the same for each trial. For Su(var)III crosses, internal controls were used. This eliminates inter-trial error and minimizes variability due to the assay system.

Using this analysis, each Su(var) can be characterized as having an enhancing, suppressing or no effect on *lt*

variegation. Enhancing effects of Su(var)s are summarized in Table 5. Many Su(var)s are capable of enhancing *lt* variegation, but Su(var)s T44 and M43 are the strongest and most general enhancers. Their effects can be seen visually and always involve 15 or more percentage unit drops in pigment from control values. Su(var)M43 strongly enhances all *ltxvs* tested, excluding *ltx18* males. Su(var)T44 strongly enhances all *ltxvs* with the exception of *ltx18* rearrangements. Su(var)B89 also has strong enhancing effects, but is more specific, affecting only *ltx2* males, *ltx24* females and *ltx6* males and females. All three of these strong enhancers belong to the unclustered group of 2L Su(var)s.

Su(var)s H69, A151 and M59 are more moderate enhancers, causing approximately 10 percentage unit drops from control pigment levels. Their effects are very specific, usually affecting less than half of the variegators tested, (see Table 5) and in most cases, they have no effect on *lt* variegation. Su(var)s B76, C119, A57 and A48 are all located on the third chromosome and are very weak enhancers of *lt* variegation, never causing pigment level drops of even 10 percentage units.

The moderate *lt* variegators, *ltx6* and *ltx2* are the most susceptable to enhancement by Su(var)s, showing reduced pigment with 7 and 9 Su(var)s tested, respectively. The strong variegating rearrangement, *ltx18* is the least susceptable, showing little reduction in pigment and no

TABLE 5: SU(VAR)S WHICH ENHANCE *LIGHT* VARIEGATION

LIGHT VARIEGATING STRAIN	SU(VAR)S WHICH ENHANCE *LIGHT* VARIEGATION	APPROXIMATE PERCENTAGE UNIT DROP IN PIGMENT (refer to figures 3a-f)
ltx18	*A151* (nonclustered 2L)	females only, 10
	M43 "	females only, 15
	M59 (clustered 2L)	females only, 9
ltx6	*T44* (nonclustered 2L)	15-25
	M43 "	20-25
	B89 "	15
	H69 (clustered 2L)	females only, 10
	B76 (3R)	males only, 5
	C119 "	males only, 7
	A57 "	males only, 5
ltx2	*T44* (nonclustered 2L)	15
	A151 "	males only, 10
	M43 "	20
	B89 "	males only, 15
	H69 (clustered 2L)	10-15
	M59 "	10-15
	A48 (3R)	males only, 7
	A57 "	males only, 3
	B76 "	females only, 6
ltx13	*T44* (nonclustered 2L)	45-50
	M43 "	15
	A48 (3R)	males only, 8
ltx24	*T44* (nonclustered 2L)	40
	M43 "	20-25
	B89 "	females only, 15
ltx4	*T44* (nonclustered 2L)	40-50
	M43 "	20
	M59 (clustered 2L)	10

response to most Su(var)s. The weak variegators *ltx13*, *ltx24* and *ltx4* were strongly affected by Su(var)s T44 and M43, but unresponsive to most other Su(var)s.

Suppression of *lt* variegation by Su(var)s is much less frequent. *ltx18* males are moderately suppressed by Su(var)C157. *ltx13* females are moderately suppressed by Su(var)B76. *ltx2* females are very weakly suppressed by Su(var)s B76 and A57 with pigment increases of aproximately 5 percentage units over control values. Similarly, *ltx6* females are very weakly suppressed by Su(var)A48. No correlation between suppression by Su(var)s and type of *ltxv* rearrangements is apparent.

DISCUSSION

The results indicate that dominant suppressors of PEV (Su(var)s) are capable of significantly enhancing the effects of heterochromatic variegating rearrangements (Figures 3a-f). Since not all Su(var)s are able to enhance *lt* variegating phenotypes, there appear to be functional differences among the Su(var)s tested. A functional difference between clustered and nonclustered mutants on the second and third chromosome has been suggested based on position only (Sinclair, *et al.*, 1983). The strongest enhancers of *lt* variegation are all nonclustered Su(var)s located on 2L. Moderate enhancers of *lt* include one nonclustered 2L Su(var) and two of the clustered 2L suppressors. These groups overlap in their abilities to enhance hPEV. In fact, one of the 2L clustered Su(var)s is capable of suppressing *lt* variegation. Four of the 3R Su(var)s can enhance *lt* variegation, but their effects are extremely weak. Although clear differences in function cannot be attributed to clustered groups, the 2L mutants as a whole have an ability to enhance hPEV while 3R Su(var)s are very weak enhancers or show no enhancing effect.

Detection of enhancement of *lt* variegation may be dependent upon the strength of the variegator being tested. Su(var)s *M43* and *T44* are general in their effects, enhancing all *lt* variegating rearrangements tested with the exception of *ltx18*. It seems likely that their mechanism of action is

similar for all *lt* variegators, but in the case of *ltx18*, detection of further inactivation through a reduced pigment phenotype is difficult. *ltx18* is an extremely strong variegator: the *light* locus is incativated in virtually all ommatidia. Therefore, the addition of a Su(var) mutation to an already drastically perturbed cell may not cause further inactivation of this locus.

In moderate and weak *lt* variegators, detection of reduced pigment is not an issue. It is surprising then, to see that the weak variegators, *ltx13*, *ltx24* and *ltx4* are less susceptible to enhancement than are moderate variegators (*ltx6*, *ltx2*). The ability of Su(var)s to enhance *lt* variegation must also be dependent upon the particular variegator present. For example Su(var)M59 enhanced *ltx4*, *ltx2* and *ltx18* variegation, but none of the other rearrangements tested. The pattern observed is difficult to associate with strength of variegation which in turn does not correlate with physical (cytological) differences between *lt* rearrangements (Hessler 1958). Differences between variegators must be due not to the relative position, but the nature of the breakpoints. Perhaps the Su(var)s are acting in a sequence dependent fashion, specific to each rearrangement.

Several possible machanisms may explain the enhancing capabilities of specific Su(var) mutations. First, a simplistic model of *lt* variegation would assume a series of events reciprocal to euchromatic variegation such as *wm4*.

That is, the *lt* gene is inactivated due to spreading of euchromatin across some boundary, or that heterochromatin is no longer maintained once a break occurs proximal to some boundary. This reciprocal model assumes that the *lt* locus requires heterochromatic packaging for proper expression. The addition of a Su(var) mutation to the variegating strain may perturb the heterochromatic environment further, causing *light* to be inactivated in a higher proportion of cells. The actual role of a suppressor mutation in this case, is to make variegation more extreme by providing fewer or aberrant elements, structural or enzymatic, necessary for normal packaging, structure and/or maintenance of heterochromatin.

A second mechanism for *lt* variegation and related Su(var) activity assumes a transvection-like model (see Lewis 1954), which involves synapse-dependent complementation of alleles. In this model, *lt* expression is dependent upon homolog (locus to locus) pairing. When one *lt* locus is rearranged, it becomes topologically difficult to pair with its homolog, thus causing variable expression from cell to cell. The role of a suppressor gene may be to facilitate proper pairing, via DNA-binding proteins or possibly through indirect (e.g. enzymatic) means. The addition of a Su(var) mutation to the variegating rearrangement would further diminish pairing, thus causing a more extreme *light* phenotype.

The results obtained do not distinguish between these models for enhancement by Su(var)s, but the first

(reciprocal) model is favored for the following reason. A transvection model predicts that a *light* rearrangement which is homozygous (such as *ltx13*) should not be a strong variegator, or perhaps should not variegate at all. As shown by basal *ltx13* pigment levels, this variegator is indeed weak. However, it responds dramatically to several Su(var)s showing significantly decreased pigment levels in the presence of Su(var)s *T44* and *M43*. This interference by the Su(var) mutation must not be a result of homolog pairing problems, since topological constraints are not a factor for this particular mutant. Therefore, a more likely role of Su(var) loci is that of heterochromatic structure or maintanence.

Su(var);*lt* combinations that do not show enhanced variegation point out that many Su(var) products appear unimportant to hPEV. These Su(var) genes may code for products which have a more general function, or functions specific to euchromatic PEV. This result also suggests that hPEV and euPEV are not simply reciprocal events. Certainly, various Su(var)s are specific to euPEV and are not involved in structures or functions crucial to the expression of a displaced heterochromatic gene.

Contrary to the models proposed above, five instances of suppression of *lt* variegation by Su(vars)s do exist in this study. None of these cases involve pigment increases of more than 13 percentage units over control values; 3 of 5 cases involve <=6 percentage unit changes. No Su(var) is

able to suppress *lt* variegation in both males and females of one genotype, and in no case is the suppression detectable visually. Su(var)*B76* both suppresses (in females) and enhances (in males) *ltx13* variegation. Su(var)*A57* also has contrasting effects on *ltx2* rearrangements. Therefore, suppression of *lt* variegation is a relatively rare, extremely weak and inconsistent event. If this suppression is a real phenomenon, these Su(var)s must have a function much different than all other Su(var)s tested here; that is, opposite to any of the proposed functions for Su(var) genes.

In conclusion, if *light* expression is dependent upon a heterochromatic environment, its expression may be disrupted in rearrangements which cannot maintain that environment. By introducing a suppressor of PEV, that gene is expected to suffer, showing enhanced variegation due to a more extreme limit on the heterochromatic environment. This effect is observed for several dominant Su(var)s, strongly suggesting that the function of these genes is essential for the appropriate expression of heterochromatic loci, but is inhibitory for euchromatic loci.

CHAPTER 2 - Characterization of a proximal cluster of Su(var) mutations on chromosome three.

INTRODUCTION

The inactivation of genes due to position effect variegation (PEV) can be attributed to chromatin changes. It follows that control of gene regulation at the chromatin level can be studied by determining the mechanisms of PEV. The investigation of dominant modifiers of PEV is one strategy used to study this process. Genetic characterization of genes involved in the process will give information on specific functions and provide valuable information towards molecular characterization of these genes. This information will contribute to our understanding of gene control at the chromatin level.

Many dominant suppressors of PEV, Su(var)s, have been isolated and characterized to varying degrees (Spofford, 1967; Reuter and Wolff 1981; Sinclair *et al.* 1983; Reuter *et al.* 1986; Reuter *et al.* 1987). Sinclair *et al.* (1983) isolated 51 dominant suppressors of PEV, Su(var)s. These mutants were mapped genetically and fell into clustered and nonclustered groups on chromosomes two and three as described in the general introduction. The clustered mutants on the left arm of chromosome two (2L) were found to be homozygous lethal, whereas clusters on the third chromosome were intially described as being homozygous viable. The latter Su(var)s make up three discrete clusters (proximal, middle and distal) on the right arm of chromosome three (3R).

Second chromosome Su(var)s have been analyzed with respect to standard modifiers of variegation (temperature and heterochromatin loss), as well as butyrate sensitivity and possible maternal effects. Clustered Su(var)s on the left arm of chromosome two (2L) are insensitive to temperature, show loss of viability when treated with butyrate (Lloyd 1986) and show no maternal effect. In contrast, non-clustered 2L mutants are temperature sensitive, are insensitive to butyrate and show a slight maternal effect. The 3R distal cluster reacts similarly to clustered 2L Su(var)s. All Su(var)s tested are sensitive to Y-chromosome (heterochromatin) loss (Sinclair *et al.* 1983; Harden 1984). Functional differences between clusters and non-clustered Su(var)s have been suggested based on these characteristics.

Reuter *et al.* (1986) characterized 63 independently isolated X-ray and ethylmethane sulfonate (EMS) induced Su(var) mutations. These mutations have been assigned to 12 separate loci mapping to chromosome three. These Su(var)s show various degrees of homozygous viability and fertility, in addition to butyrate and heterochromatin sensitivity specific to each of the 12 loci. In fact, Reuter *et al.* (1986) suggest that clusters of homozygous viable Su(var)s on chromosome three reported by Sinclair *et al.* (1983) are allelic to similar loci reported in Reuter *et al.* (1986): Su-var(3)1, Su-var(3)2 and Su-var(3)9.

This study will examine the previously uncharacterized 3R proximal cluster of Su(var) mutations. It will include nine Su(var) mutations originally assigned to this cluster, mapping between 46.4 and 54.2 and some of which may be allelic to Su-var(3)1 and/or Su-var(3)2. These dominant mutations have been physically mapped using new compound chromosome formation and deficiencies. The sensitivity to loss of heterochromatin and maternal effects has been determined, and homozygous viability and fertility have been established. Characterization using the above criteria has better defined this cluster into genetic loci and has provided information to further investigate the functions of these clustered Su(var)s.

MATERIALS AND METHODS

Stocks

All mutations have been previously described with the exception of the following. Deficiencies used to map Su(var)s and their breakpoints are listed in Table 6, followed by a cytological map of the 3R proximal region (see Figure 4). Df(3R)*e-078* was provided by Dr. Reuter and cytologically analyzed in our lab. No deletion loops were detected, so this mutation is either a very small deletion, or a point mutation (A. Dutta). C(3L)*ri*;C(3R)e^{s} is a compound chromosome strain obtained from Dr. Holm, used to map Su(var)s with respect to the centromere. Df(2R)*M-S210* (Lindsley and Grell 1968; Hilliker and Holm, 1975) is deficient for 2R heterochromatin and was used to test heterochromatic sensitivity of the Su(var)s.

Culture Conditions

As descrobed in Chapter 1.

Mapping the 3R proximal cluster Su(var)s

Attempts to map 3R proximal Su(var)s involved two methods.

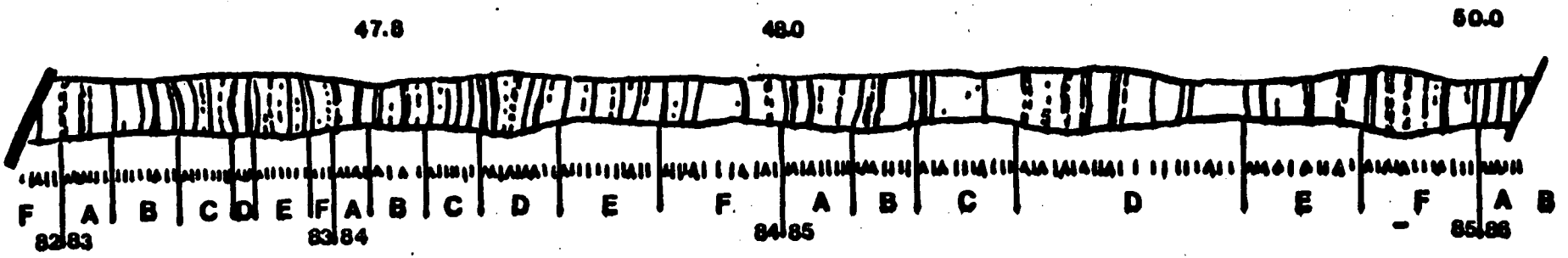
i) To map the Su(var)s into discrete regions of the chromosome, deficiencies were chosen to cover the region

TABLE 6: DEFICIENCIES USED TO MAP 3R PROXIMAL SU(VAR)S

DEFICIENCY	CYTOLOGY	REFERENCE
1. Df(3L)*Pc^{MK}*	Df(3L)78A3; 79E1,2	DIS 65, p.47
2. Df(3R)*Dfd^{+Rx13},p^{p}*	Df(3R)83E3; 84A4,5	Hazelrigg and Kaufman (1983)
3. Df(3R)*Scx^{w+Rx2},rede*	Df(3R)84A4,5; 84C1,2	"
4. Df(3R)*Scx^{w+Rx4},rede*	Df(3R)84B1,2; 84D1,2	"
5. Df(3R)*Hu^{+Rx1}*	Df(3R)84B1;2 + 84D5;84F4	"
6. Df(3R)*p^{30},rede*	Df(3R)84F4-6; 85D3-5	Kemphues *et al.* (1983)
7. Df(3R)*by^{62},rede*	Df(3R)85D11-14; 85F6 + Dp of Ant-C + more on Y	"
8. Df(3R)*E-079*	Df(3R)86E20; 87B8,9	Reuter *et al.* (1987)
9. Df(3R)*E-078*	*point mutation	Ashish Dutta
10. Df(3R)*126c*	Df(3R)87E1; F12,13	Reuter *et al.* (1987)
11. Df(3R)*kar^{sz11}*	Df(3R)87C7,8; 87E5,6	"

*originally isolated as a deficiency, this mutant was given to our lab through Dr. Speirer; cytology was done in our lab by Ashish Dutta Gupta.

FIGURE 4: CYTOLOGY OF 3R PROXIMAL REGION INCLUDING DEFICIENCIES USED TO LOCALIZE SU(VAR) MUTATIONS.



2. Df(3R) Dfd^{+Rx13}, p^{p}

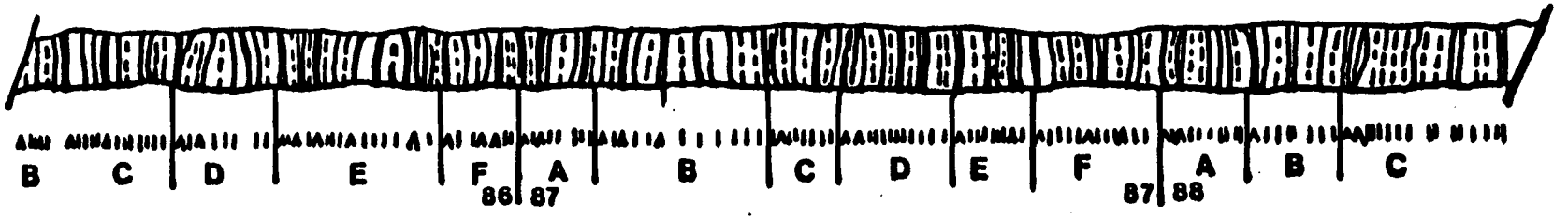
4. Df(3R) Scx^{w+Rx4}, rede

6. Df(3R) p^{30}, rede

7. Df(3R) by^{62}, rede

5. Df(3R) Hu^{+Rx1}

3. Df(3R) Scx^{w+Rx2}, rede



8. Df(3R) E-079

11. Df(3R) kar^{sz11}

10. Df(3R) 126c

9. Df(3R) E-078

proximal to the centromere on 3R since genetic mapping localized them to this area.

Most deficiencies used were rebalanced with TM3,*e,Ser* to facillitate scoring. Deficiency bearing males were first outcrossed to *wm4* virgins to check for suppressing ability which would suggest a strain deficient for a suppresssor locus. Pigment assays were performed as described previously (see Materials and Methods, Chapter 1) on *wm4;*Df flies and their *wm4;*TM3 sibs. Deficiencies acquired later from various sources were not tested by this method. Instead, the affect of the Df on *wm4* was scored visually in balancer siblings resulting from crosses described below.

Virgin Su(var)s were mated to Df/TM3 males and transferred to new food every 2-3 days. Several crosses were done at 22, 25 and 29 C, but remaining crosses were cultured at 25 C since no effect of temperature was observed. Complementation was scored by counting Df/Su(var) flies relative to their Su(var)/balancer siblings. In most cases, a minimum of 100 flies were scored where 1/3 were expected to be the diagnostic class. In non-complementation cases, the crosses were repeated so that no less than 100 flies were scored.

ii) A compound chromosome three strain, C(3L)*ri;*C(3R)e^S was used to map Su(var)s relative to the centromere of chromosome three. Two Su(var)s, *B143* and *A63*, were mapped with this method. These mutants were chosen based on their

genetic map positions as being most likely to define the outermost positions in the cluster. A summary of the protocol and strategy is given in Figure 5.

wm4; Su(var)/TM3 virgin females were collected every four hours and subjected to 2500 rads of gamma irradiation. Approximately 3300 treated virgins of each Su(var) genotype were mated after 3 days to compound males. Adults were transferred to new food three times and then discarded. Theoretically, only mutational events resulting in new compound chromosome formation and nondysjuction events will contribute to viable F1 progeny. Almost all other events will result in genetically unbalanced, and therefore inviable embryos.

Since the compound stock did not have a *wm4* rearrangement, only males show presence or absence of a suppressor directly. New compound females were tested for presence or absence of the Su(var) mutation by backcrossing to patroclinous males as shown in Figure 6. Of the *ri* progeny recovered, one half will show a *wm4* phenotype if the suppressor is not present.

Effect of heterochromatin deficiencies on Su(var) activity

Two tests were used to determine what effects alteration in the amount of genomic heterochromatin may have on the Su(var) phenotype.

FIGURE 5: STRATEGY USED TO MAP SU(VAR)S USING NEW COMPOUND FORMATION

2500 rads gamma radiation

wm4;Su(var)III x *+;C(3L)ri;C(3R)es*
TM3,Sb,Ser

↓

wm4;C(3L);C(3R) new compound formation

	NO COMPOUND	COMPOUND RIGHT	COMPOUND LEFT	NONDYSJUNCTION Su(var)/TM3 or O
ri 3L/3L		*ri* 3L/3L 3R/3R		
e^s 3R/3R			e^s 3L/3L 3R/3R	

NONDYSJUNCTION: MATROCLINOUS AND PATROCLINOUS PROGENY

3L/3L 3R/3R				* 3L/3L 3R/3R
0			3L/3R 3L/3R **	

*patroclinous progeny, *ri*, e^s phenotype like male parent
**matroclinous progeny, Su(var)/TM3 phenotype like female parent

FIGURE 6: PROTOCOL TO DETERMINE PRESENCE OR ABSENCE OF SU(VAR) IN NEWLY FORMED COMPOUND FEMALES, HETEROZYGOUS FOR *WM4*

wm4;C(3L)*ri*;*C(3R)Su? / +	x	*wm4*;C(3L)*ri*;C(3R)e^s / Y	
new compound virgin female		patroclinous male	

EXPECTED PROGENY CLASSES

PHENOTYPE:	*ri* only	e^s, *ri*
	**wm4*;C(3L)*ri*;C(3R)*Su*	*wm4*;C(3L)*ri*;C(3R)e^s
	+;C(3L)*ri*;C(3R)Su?	+;C(3L)*ri*;C(3R)e^s

*This newly formed compound chromosome possibly carries a Su(var) mutation, masked by wild type allele for *white*.

**If the Su(var) is present on C(3R), all of these progeny will be red-eyed, due to suppression of *wm4*. If some *wm4* progeny are found, Su(var) must be on 3L.

i) Loss of Y chromosome: By using a strain with the attached-X chromosome, C(1)RM,*pn*, crosses were performed to produce males containing no Y chromosome (see Figure 7). These males were compared to males which did receive a Y chromosome from an attached-X background. Both genotypes were subjected to pigment analysis as described previously. Siblings inheriting the balancer homolog serve as internal controls.

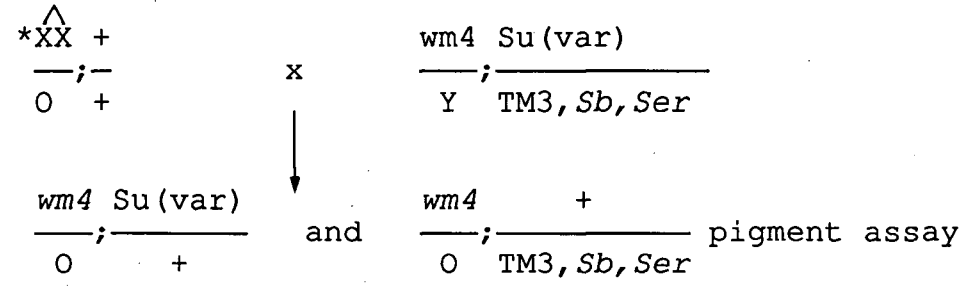
ii) Loss of 2R heterochromatin: Df(2R)*MS-210* is deficient for 2R heterochromatin only. The effect of removing this specific segment of heterochromatin on the expression of the Su(var) phenotype was examined using the protocol shown in Figure 8. Flies were raised at 25 C to ensure accurate scoring of the *Curly* wing phenotype.

Maternal Effects

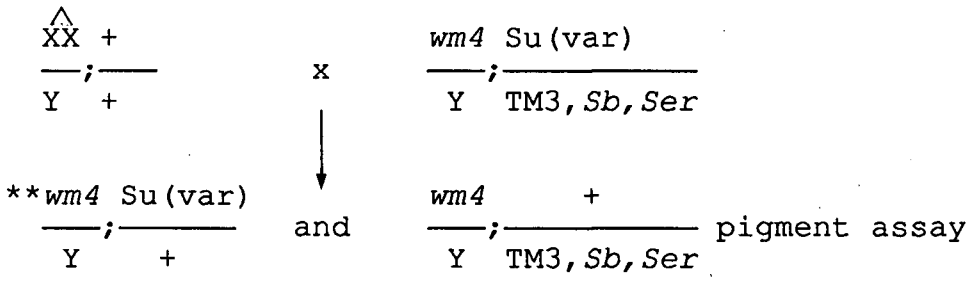
To check for maternal effects in each of the mutants, reciprical crosses were made as shown in figure 9. Temperature sensitivity was measured by carrying out the crosses at 18, 22 and 29 C. Su(var) and balancer siblings from maternal and paternal crosses raised at 22 and 29 C were compared using pigment assays as before. Crosses raised at 18 C were scored visually.

FIGURE 7: PROTOCOL USED TO DETERMINE SENSITIVITY OF SU(VAR)S TO LOSS OF THE Y CHROMOSOME

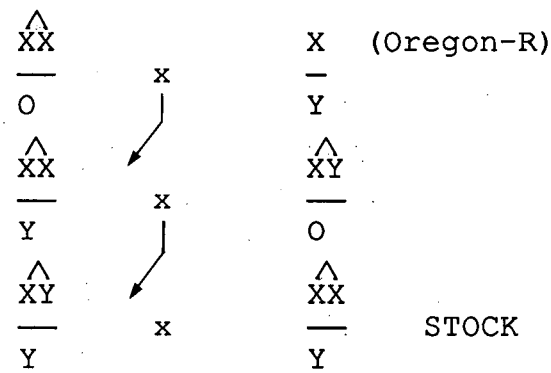
EXPERIMENTAL:



CONTROL:



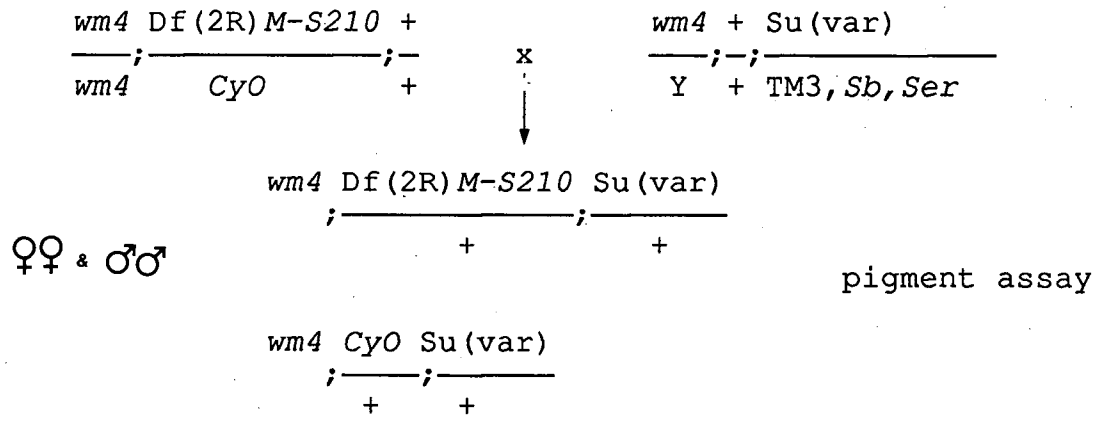
**STOCK CONSTRUCTION:



*X̂X=C(1)RM

FIGURE 8: PROTOCOL USED TO DETERMINE SENSITIVITY OF SU(VAR)S TO THE LOSS OF 2R CENTRIC HETEROCHROMATIN.

EXPERIMENTAL CROSS:



CONTROL:

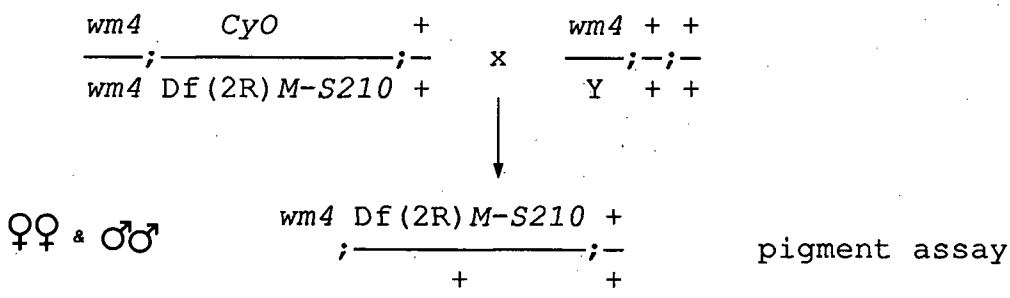
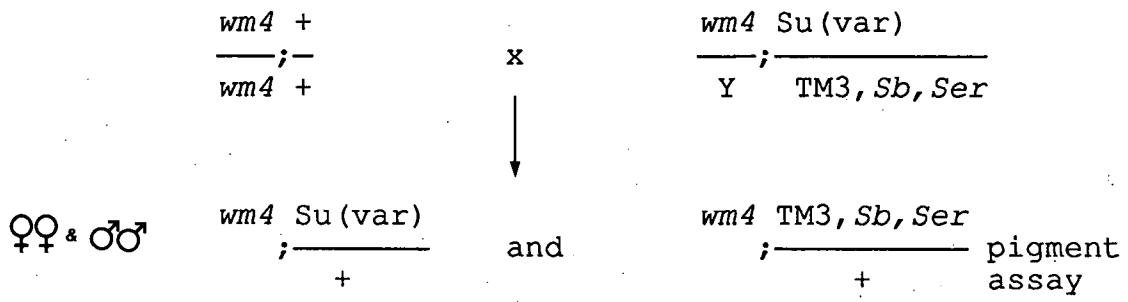
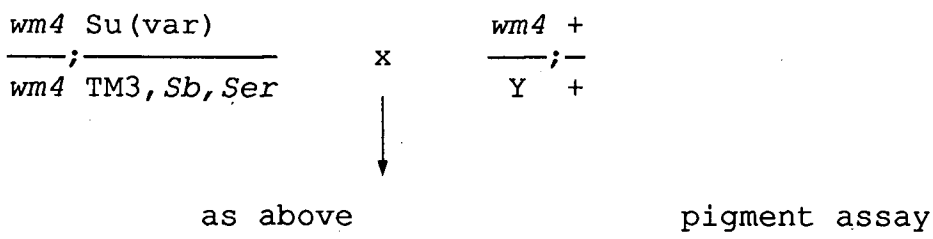


FIGURE 9: RECIPROCOL CROSSES USED TO DETERMINE MATERNAL EFFECTS FOR 3R SU(VAR)S

PATERNAL CROSS:



MATERNAL CROSS:



Viability and fertility studies/complementation tests

The first step in characterizing the cluster was to construct marked stocks of each Su(var). This was accomplished by first constructing a reliable balanced mapping strain, *wm4; Gl Sb H*/TM3,*e,Ser* and a strain with a dominant marker *Lyre* and an appropriate balancer as shown in Figures 10a and b. The cluster falls approximately 6 map units from *Glued* on the left and 8 map units from *Stubble* to its right. These strains were then used to obtain recombinant Su(var) strains with one chromosome arm marked by a dominant mutation as shown in Figure 11. Several stocks for each Su(var) were established and are listed in Table 7.

These marked Su(var) strains (M-Su(var)) were used to test for homozygous viability and fertility. Each M-Su(var) was crossed back to its original Su(var)/TM3,*Sb,Ser* stock. Strains were labeled homozygous lethal if no progeny resulted from the backcross. Semi-lethality was assumed if less that 25% of expected progeny resulted from the backcross. Surviving homozygotes resulting from this cross were allowed to mate with strains known to be fertile. If no larvae resulted, homozygous sterility was assumed.

Once homozygous phenotypes had been established, *inter se* complementation tests were done. Since it is not likely

FIGURE 10a: PROTOCOL FOR CONSTRUCTING A MULTIPLY MARKED STRAIN WITH TM3 BALANCER LACKING *STUBBLE*

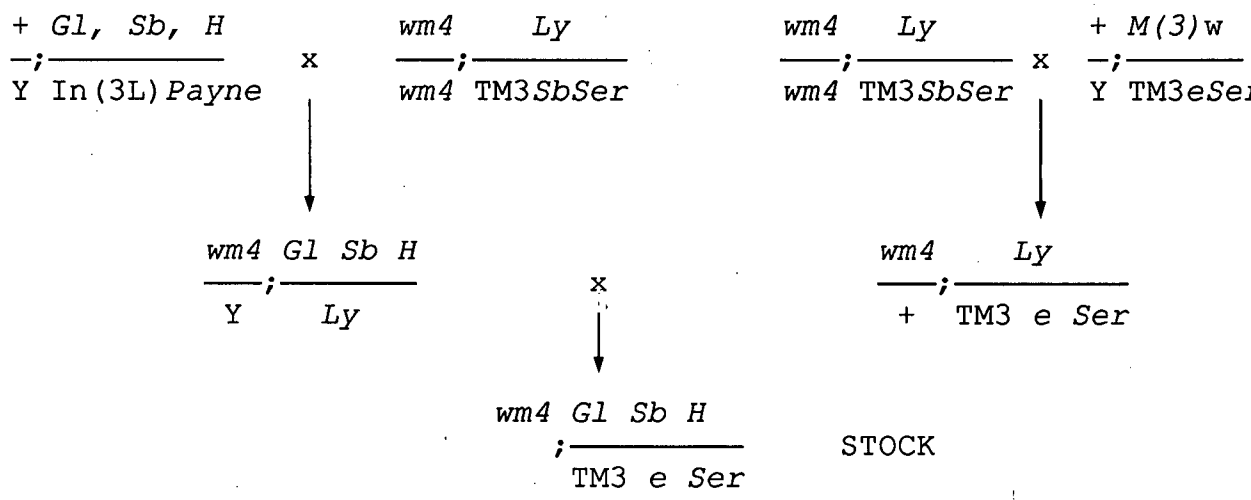


FIGURE 10b: PROTOCOL FOR CONSTRUCTION OF DOMINANTLY MARKED TM3 BALANCED STRAIN LACKING *STUBBLE*

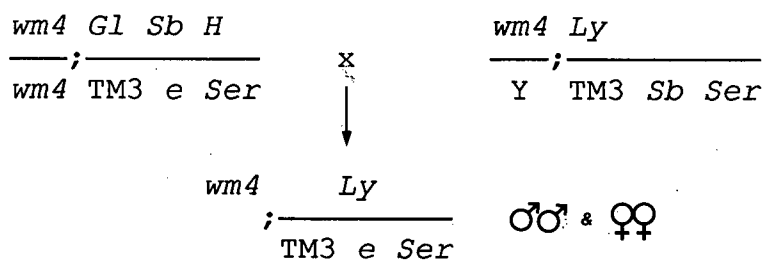
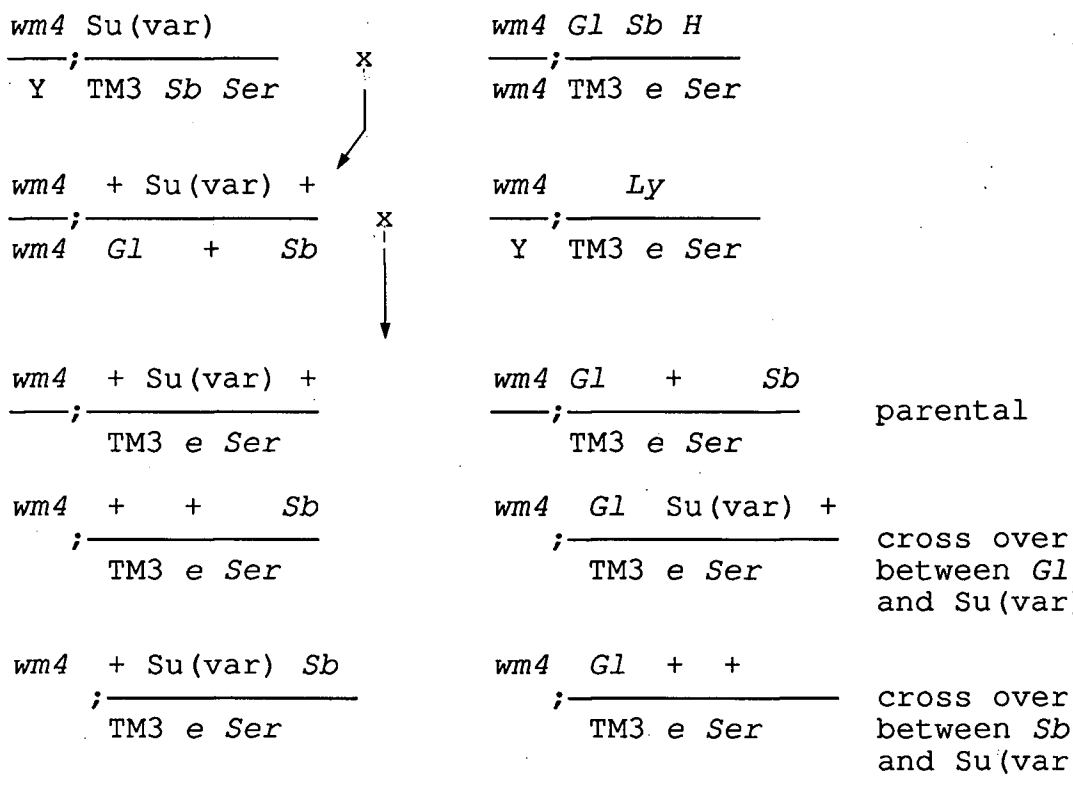


FIGURE 11: PROTOCOL FOR CONSTRUCTING MARKED STOCKS OF SU(VAR) MUTATIONS



TO ESTABLISH MARKED STOCKS:

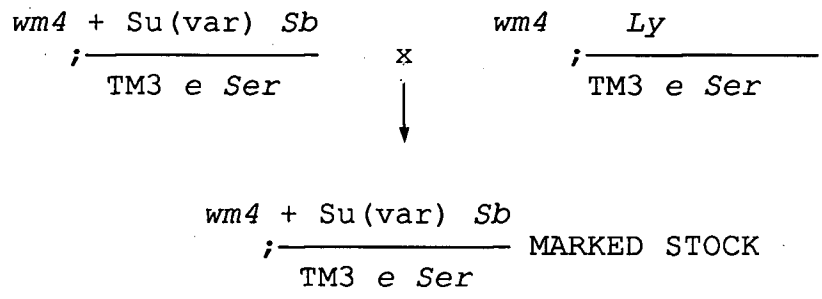


TABLE 7: MARKED STOCKS ESTABLISHED FOR SU(VAR)III MUTATIONS

M - Su(var)A63:	*C - A63, Sb*	*M - Su(var)B76:*	*A - B76, Sb*
	A - Gl, A63		*A - Gl, B76*
	B - Gl, A63		*C - Gl, B76*
	D - Gl, A63		*D - Gl, B76*
	E - Gl, A63		*E - Gl, B76*
	F - Gl, A63		*F - Gl, B76*
M - Su(var)A57:	*A - A57, Sb*	*M - Su(var)A130*	*A130 Gl Sb*
	B - A57, Sb		*A130 Gl +*
	B - Gl, A57		*A130 + Sb*
M - Su(var)B94:	*D - B94, Sb*	*M - Su(var)A160*	*C - A160 Sb*
	B - Gl, B94		*D - A160 Sb*
	E - Gl, B94		
M - Su(var)C119:	*A - C119, Sb*		
	C - C119, Sb		
	A - Gl, C119		
	C - Gl, C119		
M - Su(var)B143:	*B - B143, Sb*		
	C - B143, Sb		
	F - B143, Sb		
	A - Gl, B143		
	B - Gl, B143		
	E - Gl, B143		
M - Su(var) A48:	*B - A48, Sb*		
	C - A48, Sb		
	D - A48, Sb		
	E - A48, Sb		
	A - Gl, A48		
	B - Gl, A48		
	C - Gl, A48		

that second site lethals would map to the same positions in different Su(var)s, original Su(var)/TM3 stocks were used. Flies were mated in each pairwise combination. A minimum of 150 flies were scored for each cross. Failure to complement is seen if less than 10% expected progeny eclose and survive, or if transheterozygotes show infertility.

Statistics

Statistical tests between two mean values were carried out using an unpaired t-test (Zar 1984). Differences between 3 or more means were tested using analysis of variance (ANOVA) followed by the Neuman-Keuls multiple range test (Zar 1984). Values expressed as percentages were first transformed to their arcsine value, which converts binomially distributed data to values closely approximating a normal destribution, before statistical tests were done. Values are expressed as mean +/- standard error. $P<0.05$ was taken as the limit of significance.

RESULTS

Deficiency mapping

Deficiencies capable of suppressing *wm4* variegation are characterized by comparing *wm4*;Df/+ and *wm4*;balancer/+ siblings. Out of five deficiencies chosen for their position close to the centromere on 3R only one, Df(3R)*Scx*$^{2+Rx2}$,*red e* shows a pigment level close to that of a suppressor (see Table 8). However, its balancer siblings shows the same amount of suppression. The only case where the effects of a deficiency and its balancer are significantly different is Df(3R)*Dfd*$^{+rx13}$. In this case, the defeciency sibling has significantly less pigment than its balancer sib, suggesting that Df(3R)*Dfd* may delete a locus which enhances PEV, an En(var) locus. Deficiencies *E-079, E-078, karsz11* and *126c* show suppression according to Reuter *et al.* (1987), making these deficiencies good candidates for mapping Su(var)s. All other deficiencies tested showed no suppressing ability when observed in Df/balancer siblings of Df/Su(var) flies. Df/Su(var) heterozygotes show no abnormal eye phenotype.

Two deficiencies failed to complement with any of the Su(var)s (see Table 9). Su(var)s *A57* and *A63* are lethal in the presence of Df(3R)*E-079*. These Su(var)s likely map to the region spanned by this deficiency. Df(3R)*E-078*, which

TABLE 8: EFFECTS OF DEFICIENCIES IN THE 3R PROXIMAL REGION ON *WM4* VARIEGATION

DEFICIENCY GENOTYPE (*wm4* background)	% OREGON-R PIGMENT + S.D.	SIGNIFICANT DIFFERENCE, $p<.05$
control: *wm4*/Y; +/+	7.9 + 1.6	
Df(3R) Scx^{w+Rx4}, *rede*/+	1.7 + 0.9	no
TM3/+	0.0	
Df(3R) Dfd^{+Rx13}, p^{p}/+	9.0 + 1.9	yes
TM3/+	19.4 + 3.9	
Df(3R) Scx^{w+Rx2}, *rede*/+	48.1 + 5.3	no
TM3/+	43.7 + 5.7	
Df(3R) Hu^{+Rx1}	1.3 + 1.3	no
TM3/+	2.4 + 0.7	
Df(3R) p^{30}, *rede*/+	4.8 + 2.1	no
TM3/+	4.1 + 1.3	

TABLE 9: RESULTS OF COMPLEMENTATION ANALYSIS WITH DEFICIENCIES IN THE 3R PROXIMAL REGION AND 3R SU(VAR) MUTATIONS

	DEFICIENCY*										
Su(var)	1	2	3	4	5	6	7	8	9	10	11
B143	+	+	+	+	+	+	+	+	+	+	+
A48	+	+	+	+	+	+	+	+	+	+	+
B94	+	+	+	+	+	+	+	+	+	+	+
C119	+	+	+	+	+	+	+	+	+	+	+
B76	+	+	+	+	+	+	+	+	+	+	+
A57	+	+	+	+	+	+	+	-	-	+	+
A63	+	+	+	+	+	+	+	-	+	+	+
A160	ND	ND	ND	ND	ND	ND	+	+	+	ND	+
A130	ND	ND	ND	+	ND	+	+	+	+	ND	ND

*numbers 1-11 correspond to deficiencies listed in Table 6
+=Df/Su(var) progeny viable and fertile
-=Df/Su(var) progeny completely lethal
ND=not done

is actually a point mutation fails to complement with Su(var)*A57*, but not *A63*, suggesting that these two Su(var)s are separable loci. Since these deficiencies possibly failed to complement with a second site lethal, crosses were repeated with marked stocks of Su(var)s *A57* and *A63*. Identical results were found, suggesting that Su(var)s *A63* and *A57* actually map in the region of 86E-87B.

Compound Mapping

New compound chromosome formation was undertaken to assign locations for Su(var)*A63* and *B143* to either side of the centromere. Flies with either *the* e^S or *ri* phenotype, but not both, indicated a new compound formation. Since the marked compound stock did not carry a *wm4* rearrangement, the presence or absence of a suppressor could be seen directly only in the males. Su(var)s tested were effectively homozygous lethal (possibly due to second site lethals) so the occurance of a *wm4* male with a new compound must be used indirectly to assign the Su(var) to 3L or 3R.

Su(var)*B143* crosses yeilded 5 new compound formations (see Table 10) including one *ri* male which was immediately informative. Since the male was *wm4;* C(3R);C(3L)*ri* and shows a *wm4* phenotype, Su(var)*B143* is not on 3R. A female of the same phenotype was progeny tested and found to be Su(var)+, confirming that Su(var)*B143* maps to 3L (see Table 11). Three e^S females recovered had the genotype *wm4*/+;

TABLE 10: SUMMARY OF NEW COMPOUND PROGENY RECOVERED FROM GAMMA RADIATION SCREEN

SU(VAR) SUBJECTED TO GAMMA TREATMENT	GENOTYPE OF NEW COMPOUND PROGENY C(3L);C(3R)e^s	C(3L)*ri*;C(3R)	LOCATION
Su(var)A63 / TM3 *Sb Ser*	4 males, *wm4* phenotype 5 females, wild type eye non-virgins	no progeny	3R
Su(var)B143 / TM3 *Sb Ser*	3 females, sterile	1 male, *wm4* phenotype 1 female, wild type eye *progeny tested	3L

*progeny test results shown in Table 11

TABLE 11: RESULTS OF PROGENY TESTING FOR NEW COMPOUND FEMALES HETEROZYGOUS FOR *WM4;* TEST FOR PRESENCE OR ABSENCE OF *SU(VAR)B143.*

GENETIC CROSS: $\underline{wm4}$;C(3L)*ri*;C(3R) x $\underline{wm4}$;C(3L)*ri*;C(3R)e^s
+ Y (patroclinous male)

RESULTING PROGENY PHENOTYPE:	*ri* only	*ri*, e^s
	wm4;C(3L)*ri*;C(3R)	*wm4*;C(3L)*ri*;C(3R)e^s
	3 wm4 males 2 wm4 females	4 wm4 males 5 wm4 females
	+;C(3L)*ri*;C(3R)	+;C(3L)*ri*;C(3R)e^s
	6 males 3 females	4 males 3 females

If Su(var) is present on C(3R), all *ri* only progeny will have red eyes. If some *ri* only progeny are present with *wm4* eyes, the Su(var) must not be present.

Since *wm4, ri* progeny result from the cross, *Su(var)B143* is not present on C(3R) and must be on 3L.

C(3L)*B143*;C(3R)e^{S}. They were all sterile, which is expected based on spontaneous B143 homozygotes observed in stock.

Nine new compound formations were recovered from Su(var)*A63* crosses (see Table 10). All males were of the genotype *wm4*;C(3L)/C(3R)e^{S}. Since they were all *white* mottled, Su(var)*A63* can be assigned to the right arm of chromosome three. Females recovered were not virgins, so progeny testing was not done.

Effect of heterochromatin loss on Su(var) activity

i) C(1)RM,*pn*/O females were crossed to Su(var)/TM3 males to produce male progeny deficient for the Y-chromosome. Pigment levels for these flies are shown in Table 12. These X/O; Su(var) male flies are compared to X/Y; Su(var) flies from a cross which controls for any effects of C(1)RM,*pn* background.

The loss of a Y chromosome has a dramatic and significant effect on 8 of 9 Su(var)s tested. Su(var)A130 is the only strain which shows no enhancement of variegation due to loss of the Y chromosome. It is also one of the mutants found to map outside the cluster (see appendix). However, the other non-clustered suppressor tested, Su(var)*A160* shows a large decrease in pigment, almost down to *wm4* levels. All other Su(var)s are strongly affected by loss of a Y-chromosome. These results are consistent with

TABLE 12: EFFECTS OF LOSS OF Y CHROMOSOME ON SU(VAR) MUTATIONS OF CHROMOSOME 3

	X/O; SU(VAR) values are % of Oregon-R pigment levels	X/Y; SU(VAR)	t-value
wm4/O	2.5 ± 0.3	4.6 ± 1.1	4.9
wm4;Su(var)			
B76	69.0 ± 7.8	105.1 ± 3.2	11.4
A48	12.0 ± 1.8	71.0 ± 6.3	21.2
B94	20.9 ± 5.0	89.9 ± 7.9	11.7
C119	13.7 ± 0.5	83.2 ± 9.5	31.1
A63	5.1 ± 0.2	49.4 ± 12.1	32.5
A57	14.1 ± 1.6	58.9 ± 10.4	13.7
B143	16.7 ± 2.4	60.3 ± 5.2	15.9
A130	103.3 ± 2.3	103.8 ± 2.9	0.3
A160	14.4 ± 3.0	74.8 13.8	10.8

Values given are mean ± S.D.; critical t-value, df(8)=2.31

those reported by Sinclair *et al.*(1983) and Harden (1984) for all Su(var)s studied, excepting Su(var)*A130*.

ii) Df(2R)*M-S210* is known to enhance variegation (Morgan *et al.* 1941) and is deficient for centromeric heterochromatin on the right arm of chromosme two (Hilliker and Holm 1975). Although this deficiency is also *Minute* in phenotype, this mutation has been shown to have no significant effect on variegation of *wm4* (Harden 1984). Flies were raised at 25 C and this is reflected by the relatively high *wm4* control values shown in Table 13.

Df(2R)*MS-210* effects *wm4* variegation at this temperature, causing an enhanced phenotype or reduction in pigment levels. In addition, Df(2R)*MS-210* significantly reduces pigment in 8 of 9 suppressors tested. The enhancing cabability of Df(2R)*MS-210* is much weaker than that caused by loss of the Y-chromosome, but is consistent throughout. Again, only Su(var)*A130* is completely unaffected by loss of heterochromatin. In general, both males and females are affected by Df(2R)*MS-210*, but females appear more susceptable to the heterochromatin loss. Where both sexes are influenced, females invariably show a larger difference in comparing *Minute* individuals to their *CyO*-balancer siblings. The exception is Su(var)*B76*, where only males are significantly different than their *CyO* balancer sibs.

These results are consistent with the findings of Reuter *et al.* (1983) which show the suppressors on

TABLE 13: EFFECTS OF LOSS OF 2R HETEROCHROMATIN ON SU(VAR) MUTATIONS ON CHROMOSOME 3

		Df(2R)*M-S210*;Su(var) values are % Oregon-R pigment + S.D.	*CyO*;Su(var)	t-value
wm4;+	male	9.6 ± 0.5	17.4 ± 1.7	t(8)=12.3
	female	9.9 ± 1.0	40.3 ± 2.7	t(8)=23.7
B76	male	69.4 ± 4.5	89.9 ± 3.8	t(8)=6.6
	female	72.5 ± 6.8	83.0 ± 6.8	t(7)=2.0*
C119	male	56.9 ± 4.1	70.1 ± 3.4	t(8)=5.0
	female	50.3 ± 1.4	71.6 ± 4.6	t(8)=10.8
A63	male	61.8 ± 5.5	91.1 ± 6.1	t(8)=6.5
	female	58.8 ± 4.0	85.8 ± 4.7	t(8)=8.6
B143	male	67.0 7.8	81.3 6.6	t(8)=3.2
	female	41.7 ± 5.1	76.7 ± 3.5	t(8)=11.8
A48	male	58.6 ± 10.0	85.3 ± 8.9	t(8)=4.1
	female	41.1 ± 4.2	81.1 ± 6.6	t(8)=10.1
A57	male	70.7 ± 4.4	108.3 ± 10.0	t(8)=9.0
	female	54.7 ± 6.4	96.1 ± 6.6	t(8)=8.2
B94	male	59.4 ± 7.4	94.6 ± 8.9	t(8)=5.4
	female	45.9 ± 5.3	88.1 ± 2.1	t(8)=16.3
A160	male	52.1 ± 5.8	90.2 ± 5.7	t(8)=7.9
	female	36.9 ± 2.9	80.2 ± 9.5	t(8)=10.6
A130	male	69.6 ± 1.5	73.9 ± 4.8	t(8)=1.5*
	female	70.5 ± 3.6	72.1 ± 3.6	t(8)=0.6*

t-values are given with degrees of freedom in (); critical t-value, df(8)=2.31; df(7)=2.37
*insignificant difference between Df(2R)*M-S210* and *Cyo* progeny.

chromosome 2 and 3 are affected by removal of 2R heterochromatin. However, the distal cluster Su(var)s on chromosome 3 have been tested (Harden, 1984) and show no enhancement by this same deficiency.

Maternal effects

To determine whether the Su(var) mutations had any maternal effect on variegation, reciprocal crosses were made. The variegating non-Su(var) offspring from each cross were examined for the amount of eye pigment. Pre-zygotic expression of maternal RNAs may be detected as a suppressed phenotype in *wm4;*+/TM3 (Su+) progeny of Su(var) female parents. To test for temperature sensitivity of the product, reciprocal crosses were done at 18, 22 and 29 C. Results of the crosses are presented in Tables 14 and 15. Control *wm4* variegating levels are normal for 22 and surprisingly higher at 29 C. Females are more susceptable to suppression caused by high temperature in this *wm4* strain (also observed by Harden, 1984). Although *wm4;*+/TM3 levels are often lower than control values, any influence of the TM3 balancer does not interfere with comparison of maternal and paternal progeny, since both carry the balancer. Su(var)/+ progeny have pigment values within the normal ranges observed.

At 18 C, visual observations suggested no differences between maternal and paternal crosses (data not shown).

TABLE 14: MATERNAL EFFECTS MEASURED AT 22 C

GENOTYPE (*wm4* background)	SEX	PATERNAL CROSS values are % Oregon-R pigment ± S.D.	MATERNAL CROSS values are % Oregon-R pigment ± S.D.	t-value
B143/+	male	54.9 ± 5.4	43.4 ± 5.1	t(8)=3.1*
	female	32.1 ± 7.9	47.9 ± 6.4	t(8)=3.1*
TM3/+	male	19.8 ± 3.3	7.6 ± 1.7	t(7)=6.6*
	female	5.8 ± 1.7	13.0 ± 3.3	t(8)=4.2*
B76/+	male	75.5 ± 9.0	82.6 ± 4.4	t(8)=1.5
	female	75.6 ± 4.4	70.7 ± 10.1	t(7)=0.9
TM3/+	male	4.4 ± 1.5	9.2 ± 1.6	t(8)=4.5*
	female	3.5 ± 2.4	7.9 ± 2.5	t(7)=2.4*
A57/+	male	69.1 ± 4.9	36.8 ± 2.9	t(8)=12.3*
	female	54.1 ± 9.5	52.5 ± 5.1	t(8)=0.4
TM3/+	male	20.5 ± 5.0	8.8 ± 3.0	t(8)=3.8*
	female	15.8 ± 5.6	14.8 ± 3.3	t(7)=0.2
A48/+	male	76.3 ± 3.1	43.8 ± 6.1	t(8)=10.2*
	female	54.8 ± 6.8	56.9 ± 2.5	t(8)=0.7
TM3/+	male	15.7 ± 1.4	5.3 ± 1.8	t(5)=6.9*
	female	9.3 ± 3.4	17.4 ± 2.7	t(8)=3.9*
B94/+	male	75.1 ± 2.9	55.6 ± 2.8	t(8)=5.9*
	female	57.3 ± 5.8	68.1 ± 4.7	t(8)=3.1*
TM3/+	male	15.8 ± 4.7	7.8 ± 5.9	t(8)=0.9
	female	6.7 ± 3.8	37.7 ± 6.0	t(8)=8.6*
A63/+	male	71.4 ± 9.1	50.9 ± 5.4	t(8)=4.3*
	female	52.5 ± 7.7	64.8 ± 3.2	t(8)=3.4*
TM3/+	male	10.9 ± 2.4	11.7 ± 3.7	t(8)=1.2
	female	5.1 ± 2.6	15.2 ± 2.4	t(8)=5.8*
C119/+	male	65.6 ± 5.2	67.5 ± 2.4	t(8)=0.8
	female	50.7 ± 5.2	67.3 ± 3.1	t(8)=5.6*
TM3/+	male	15.6 ± 3.2	14.8 ± 1.2	t(8)=0.5
	female	5.7 ± 1.1	27.2 ± 5.9	t(8)=10.4*
A130/+	male	79.4 ± 1.2	65.4 ± 6.6	t(8)=7.0*
	female	72.1 ± 7.6	71.8 ± 8.3	t(8)=0.0
TM3/+	male	9.4 ± 0.9	5.4 ± 1.5	t(8)=4.8*
	female	3.3 ± 1.4	14.0 ± 2.0	t(8)=8.6*
A160/+	male	66.7 ± 5.8	62.7 ± 3.7	t(8)=1.2
	female	50.8 ± 5.7	58.9 ± 8.0	t(8)=1.7
TM3/+	male	18.1 ± 3.3	8.4 ± 2.1	t(8)=5.0*
	female	12.4 ± 3.4	10.9 ± 3.0	t(8)=3.0

wm4 control values: males, 7.0 ± 2.2; females, 9.2 ± 2.3.
t-values are given with degrees of freedom in (); critical t-values: df(8)=2.31, df(7)=2.37, df(5)=
*significant difference between maternal and paternal progeny

TABLE 15: MATERNAL EFFECTS MEASURED AT 29 C

GENOTYPE (*wm4* background)	SEX	PATERNAL CROSS values are % Oregon-R pigment ± S.D.	MATERNAL CROSS values are % Oregon-R pigment ± S.D.	t-value
B143/+	male	50.5 ± 4.8	47.8 ± 3.2	t(8)=0.9
	female	58.7 ± 4.5	56.1 ± 6.2	t(8)=0.7
TM3/+	male	19.7 ± 6.3	13.2 ± 4.7	t(8)=1.6
	female	20.5 ± 3.6	34.1 ± 9.9	t(8)=3.2*
B76/+	male	82.2 ± 4.4	93.0 ± 3.5	t(8)=3.5*
	female	91.4 ± 6.2	96.0 ± 4.7	t(8)=1.3
TM3/+	male	9.7 ± 1.4	17.3 ± 3.5	t(8)=4.9*
	female	32.4 ± 4.2	55.0 ± 5.9	t(8)=5.7*
A57/+	male			
	female	see Table 17		
TM3/+	male			
	female			
A48/+	male	59.0 ± 7.8	63.9 ± 6.2	t(8)=5.4*
	female	73.4 ± 5.8	85.4 ± 6.1	t(8)=2.2
TM3/+	male	16.0 ± 4.6	16.3 ± 2.7	t(8)=0.2
	female	24.6 ± 2.5	41.1 ± 6.1	t(8)=5.6*
B94/+	male	60.7 ± 1.9	78.8 ± 2.0	t(8)=13.4*
	female	67.4 ± 6.2	95.0 ± 1.6	t(6)=9.2*
TM3/+	male	19.6 ± 1.7	35.8 ± 6.1	t(7)=7.0
	female	26.0 ± 6.5	71.8 ± 6.9	t(8)=9.0*
A63/+	male	79.9 ± 5.0	94.8 ± 4.6	t(8)=4.4*
	female	91.3 ± 5.2	102.2 ± 1.2	t(8)=6.9*
TM3/+	male	25.0 ± 3.1	28.4 ± 6.6	t(8)=1.0
	female	34.9 ± 5.4	67.3 ± 4.6	t(7)=8.3*
C119/+	male	89.5 ± 4.8	80.2 ± 10.6	t(8)=1.8
	female	100.0 ± 2.0	97.5 ± 1.2	t(8)=3.5*
TM3/+	male	29.7 ± 6.8	35.6 ± 5.8	t(8)=1.3
	female	36.5 ± 5.4	62.0 ± 7.4	t(8)=5.7*
A130/+	male	77.2 ± 4.6	84.6 ± 4.3	t(7)=2.1
	female	80.7 ± 6.0	86.4 ± 3.7	t(8)=1.6
TM3/+	male	14.5 ± 2.4	15.4 ± 2.3	t(8)=0.6
	female	21.5 ± 5.5	47.9 ± 6.4	t(8)=6.2*
A160/+	male	59.3 ± 5.1	36.9 ± 2.2	t(8)=9.2*
	female	78.4 ± 10.6	70.8 ± 3.9	t(8)=1.9
TM3/+	male	32.0 ± 9.1	14.7 ± 4.8	t(8)=3.7*
	female	53.0 ± 4.4	36.0 ± 7.2	t(8)=4.2*

wm4 control values: males, 12.0 ± 3.4; females, 46.6 ± 4.9.
t-values are given with degrees of freedom in (); critical t-values: df(8)=2.31, df(7)=2.37, df(6)=2.45
*significant difference between maternal and paternal progeny

Flies raised at 22 C show sexual dimorphisms among *wm4;* TM3/+ (Su+) progeny (see Table 16 for summary). Differences between maternally and paternally derived progeny are small, but statistically significant. Seven of nine maternally derived female *wm4;*+/TM3 progeny show higher pigment levels than their paternally derived counterparts. This is recognized as a maternal effect. The maternal effect is observed only in the female progeny except for the case of Su(var)*B76,* in which both males and females show a maternal effect. Su(var)s *B94* and *C119* are most sensitive to maternal effects, with maternally derived +/TM3 females having pigment levels greater than 20 percentage units over paternally derived females. Su(var)*A160* and *A57* females are insensitive to maternal effects at this temperature.

Males show a different trend. Five out of nine Su(var)s show *wm4;*+/TM3 males from paternal crosses with pigment levels significangly higher than analogous maternally derived males. The suppression seen in these Su(var)+ males is labelled a paternal effect. The paternal effects observed are weaker than maternal effects seen in females; Su+ females show relatively high pigment levels compared to Su+ males and females of paternal crosses, as well as to *wm4* control values at this temperature. Su(var)*B94, A63* and *C119* males are not affected by paternal factors. An exception, Su(var)*B76* crosses show Su(var)+ paternally derived males with significantly lower pigment

TABLE 16: SUMMARY OF MATERNAL AND PATERNAL EFFECTS OF *WM4*;TM3 PROGENY OF SU(VAR) PARENTS AT 22 AND 29 C

SU(VAR) PARENT	22 C MALE	22 C FEMALE	29 C MALE	29 C FEMALE
A57	PA	NE	SEE FIGURE 17	
A48	PA	MA	NE	MA*
B94	NE	MA*	MA*	MA*
A63	NE	MA	NE	MA*
C119	NE	MA*	NE	MA*
B143	PA	MA	NE	MA
B76	MA	MA	MA	MA*
A130	PA	MA	NE	MA*
A160	PA	NE	PA	PA*

PA=paternal effect, MA=maternal effect, NE=no effect.
*difference between maternal and paternal progeny was greater than 15 percentage units.

levels than their maternal analogs. In this case, both males and females exhibit a maternal effect.

At 29 C, 8 of 9 Su(var) crosses have females which demonstrate a maternal effect. The strongest effects with respect to pigmentation are observed in Su(var)s *A130, B94, A63* and *C119*, with pigment differences greater than 25 percentage units between maternally and paternally derived Su+ females. Maternal effects are noticebly stronger at 29 C, often with pigment levels approximately 10 percentage units over 22 C levels.

The most interesting case is that of Su(var)*A57*. Initially, this suppressor was labeled temperature sensitive maternal lethal since no maternally derived progeny were recovered at 29 C after two attempts. The crosses were repeated with egg lays at 22 and 29 C, but rearring at 29 C. Special care was taken to avoid dessication and overcrowding. Maternally derived progeny can survive, but at much lower frequencies than paternally derived progeny (see Table 17). Survival is lowest for progeny maintained at 29 from oogenesis through pupation. A strong maternal effect is observed in females, but only when oogenesis takes place at 29 C. These females appear completely suppressed. (Flies were scored visually since pigment assays require a minimum of 25 flies per genotype).

Su(var)*A160* is an exception to the female specific maternal effect. Both males and females in this reciprocal cross exhibit a paternal effect. This is fairly consistent

TABLE 17: MATERNAL EFFECTS OF *SU(VAR)A57* AT DIFFERENT DEVELOPMENTAL TEMPERATURES

DEVELOPMENTAL TEMPERATURE	PATERNAL				MATERNAL			
	TM3/+		*A57*/+		TM3/+		*A57*/+	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
GROUP I (4 vials) no. of progeny:	30+	30+	30+	30+	3	3	3	10
phenotype:	*wm4*		suppressed		*wm4*		suppressed	
GROUP II (8 vials) no. of progeny:	30+	30+	30+	30+	4	5	4	9
	30+	30+	30+	30+	3	6	0	16
phenotype:	*wm4*		suppressed		*wm4*	suppressed (80%)	suppressed	
GROUP III (4 vials) no. of progeny:	30+	30+	30+	30+	0	2	1	1
phenotype:	*wm4*		suppressed		supressed (80%)		suppressed	

GROUP I: Oogenesis @ 22 C, food @ 22 C, developmental temp. 29 C
GROUP II: Oogenesis @ 29 C, food @ 22 C, developemntal temp. 29 C
GROUP III:Oogenesis through pupation, 29 C.
Phenotypes were scored visually, estimated suppression given in ().

with observations from 22 C where males showed a paternal effect and females showed no effect. Males tend to show no susceptability to parental effects at 29 C. Exceptions in addition to Su(var)*A160* include Su(var)*B76*, where both males and females show maternal effects as at 22 C; and Su(var)*B94* where males show a maternal effect.

Homozygous viability/complementation analysis

Premliminary experiments suggested that many of the third chromosome proximal cluster of Su(var)s were homozygous viable. The lethality observed was likely due to second site lethal mutations. However, no homozygous lines were established, suggesting that homozygotes while viable, may have been very weak or sterile. To determine homozygous viability and fertility, marked stocks were constructed with the intent of crossing off any second site lethals. In the process of establishing recombinant lines, the Su(var) mutations were remapped. It became apparent that two of the Su(var)s were wrongly assigned to the cluster. Su(var)*A130* and *A160* map to the left of *Glued* and *Stubble*, that is in a more distal location on 3L (see appendix). These mutants were characterized along with the proximal cluster, and used as a comparison for nonclustered verses clustered Su(var)s.

These marked stocks (refer to Table 7) were crossed to original Su(var)/TM3,*Sb,Ser* stocks to look for homozygous viable and fertile progeny. Results are given in Table 18.

TABLE 18: HOMOZYGOUS PHENOTYPES OF PROXIMAL 3R SU(VAR)S

SU(VAR)	*LETHAL	SEMI-LETHAL	VIABLE	STERILE
A63	X			
A48	X			
B143		X		
B76			X	
C119	X			
A57	X	X**		
B94	X			
A160	X			
A130			X	X

*Lethality, semi-lethality and sterility are defined in Materials and Methods, p.52.

**one recombinant strain, *B - G1, A57*, showed 3 female survivors out of 194 *A57*/TM3 siblings. These females were fertile.

Among the 9 Su(var)s tested, five remained homozygous lethal, even with both chromosome arms crossed off. Of the three homozygous viable Su(var)s, only one, Su(var)*B76* is fertile. This Su(var) strain is maintained as a homozygous stock. Su(var)*B143* is semi-lethal. Few flies survive (20% of expected based on Su(var)/TM3 siblings) and these are sterile. This is consistent with the observation that homozygotes which appear within this TM3-balanced stock are sterile (personal observations). Su(var)*A130* is completely viable when homozygous, but is sterile. Su(var)*A57* is homozygous lethal, except for one strain, *B-G1 A57*. Surviving flies are fertile.

Since many Su(var)s exhibit phenotypes such as homozygous or hemizygous lethality or sterility, *inter se* complementation tests were done. Matings were set up in every pairwise combination. Results are summarized in Table 19. Su(var)s making up the 3L complementation group show a spread-wing phenotype as trans-heterozygotes, along with complete sterility. This wing phenotype has been reported for Su(var) homozygotes mapping to this region, characterized by Reuter *et al.* (1986) and for 2L Su(var) trans-heterozygotes (personal communication, Jo-Ann Brock).

TABLE 19: COMPLEMENTATION ANALYSIS OF 3R PROXIMAL SU(VAR) MUTATIONS

SU(VAR) STRAIN	*A63*	*A48*	*B143*	*B76*	*C119*	*A57*	*B94*	*A160*	*A130*
A63	--	+	+	+	+	+	+	+	+
A48		--	FS,W	+	FS,W	+	FS,W	+	+
B143			--	+	FS,W	+	FS,W	+	+
B76				--	+	+	+	+	+
C119					--	+	FS,W	+	+
A57						--	+	+	+
B94							--	+	+
A160								--	+
A130									--

+=full complementation, FS=female sterile, W=spreadwing phenotype in male trans-heterozygotes.

DISCUSSION

The 3R proximal cluster of Su(var) mutations maps between 46.4 + 1.1 and 54.4 + 0.7 map units. Since the centromere is assigned a map position of 46.0 (Lindsley and Grell 1968) it was possible that these mutations spanned the centromere. Mapping by compound autosome formation has split this genetic cluster into at least two loci with one on 3L and the other on 3R.

Deficiency mapping extends the cluster distally, out to 87B. Su(var)A63 fails to complement with Df(3R)E-079, but complements Df(3)E-078 while Su(var)A57 is lethal with both deficiencies. As mentioned previously, according to cytogenetic analysis carried out in this lab, Df(3)E-078 is actually a point mutation. Therefore, Su(var)*A63* and Su(var)*A57* probably represent two separate loci. Their positions correspond roughly to those of *Su-var(3)13* and *Su-var(3)6* isolated and mapped by Reuter *et al.* (1986, 1987). *Su-var(3)6* and *13* are recessive lethals as are both Su(var)*A63* and *A57*, strongly suggesting these four suppressors are allelic pairs. This could be proved with complementation tests between these Su(var) alleles. Such tests would also confirm that lethality is not due to second site mutations, since these would not likely map to identicle loci among these mutants.

Loss of the Y-chromosome significantly reduces pigment in all Su(var)s previously tested (Sinclair *et al.* 1983;

Harden 1984). In eight of nine Su(var)s tested in this study, variegation was enhanced dramatically by Y-chromosome loss and was similarily and significantly affected by deficiency of 2R heterochromatin. This is in contrast to the behaviour of distally clustered Su(var) loci which show no sensitivity to 2R heterocromatin loss (Harden 1984). Reuter *et al.* (1983) reported a third chromosome suppressor, *Su(var)c*^{1001}, which was greatly affected by Df(2R)*MS-2*^{10}. *Su(var)c1001* maps to 46.7, within the 3R proximal cluster, suggesting allelism to one or more of the Su(var) genes in this study. Again, complementation tests could determine their relationships.

Su(var)*A130* is a surprising exception to the high heterochromatin sensitivity exhibited by most Su(var)s. This suppressor is not affected by loss of Y chromosome or 2R heterochromatin. It is a non-clustered suppressor which maps near the tip of 3L. This insensitivity to heterochromatin suggests a functional difference between Su(var)*A130* and other Su(var) genes. It has been hypothesized that loss of the heterochromatic Y chromosome frees heterochromatic elements which are then available to inactivate genes through position effect variegation in a higher proportion of cells, thus enhancing variegation (Zuckerkandl 1974). Su(var)*A130* may be so strong that no amount of free heterochromatic elements can make up for the deficiency of Su(var)*A130* product. Alternately, Su(var)*A130* may produce a non-structural product involved in the control

of gene inactivation in PEV. For example, this product may act to maintain structural decisions. If early on, the *white* gene was packaged as heterochromatin, but no signal (suppressor product) was available to maintain this facultative packaging, the *white* gene inactivation may be reversed and that decision clonally maintained, resulting in a suppressed phenotype. This type of developmental model could be investigated if temperature sensitive phenotypes were established.

Reciprocal crosses among the Su(var)s revealed that some (including Su(var)*A130*) have female-specific, temperature sensitive maternal effects. These suppressors must act early in development, before zygotic transcription begins, and produce a protein product, subject to heat denaturation (inactivation). By doing shifts from permissive to restrictive temperatures, developmental activity of the Su(var)s can be determined. It has already been determined that Su(var)*A57* acts extremely early, during oogenesis. Eggs laid at 22, but shifted to 29 C after 24 hours do not show the maternal suppression or the lethality observed in this strain when raised at constant (29 C) temperature.

Paternal and maternal effects could also be due to some sort of chromosome imprinting. Chromosomes from paternal or maternal Su(var) parents could be pre-programmed by being in this genetic background, and therefore exhibit a Su(var) phenotype even if they are genotypically Su(var)$^{+}$.

Genetic characterization is crucial to understanding the functions of suppressor loci. By establishing homozygous viability, lethality and sterility, it becomes possible to determine the genetic makeup of the cluster. Homozygous viability tests show that most of the Su(var)s mapping to the cluster are homozygous lethal or sterile as marked stocks. Only Su(var)*B76* is homozygous viable and fertile. The possibility exists that second site lethals may remain on those portions of chromosome not replaced by recombination. However, it seems relatively unlikely that all five homozygous lethal stocks carry a second site lethal mutation between the 2 markers *Glued* and *Stubble*. This region makes up an absolute maximum of 15% of the chromosome, if the recombination breakpoints were located exactly proximal to either marker. Furthermore, two Su(var) loci are homozygous viable or semi-lethal, but sterile. This sterility must be separate from the lethality observed in the original balanced stocks.

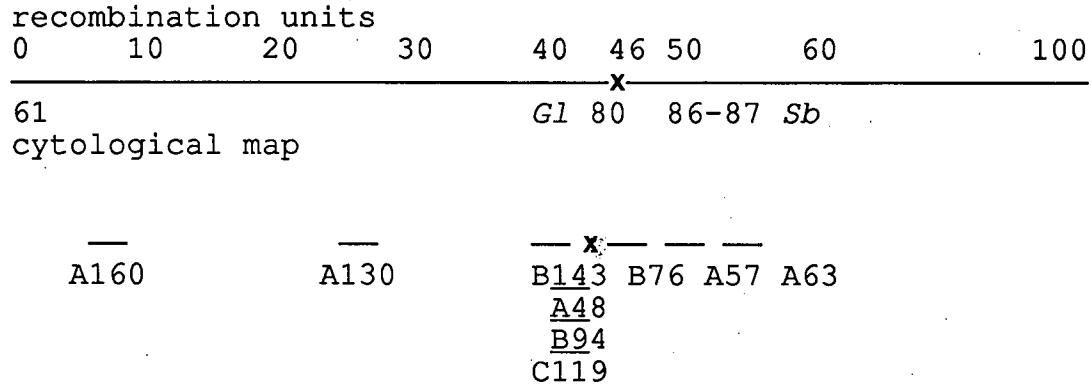
The assumption of second site lethals has been discussed by Nash *et al.* (1983). Their concern is that EMS mutagenesis is quite effective at inducing "many" extraneous lethal mutations which may mask a haplo-specific lethal mutation by exhibiting homozygous lethality not due to the mutation in question. They define a locus as being haplo-specific lethal if it is lethal over a deficiency , but not lethal as a homozygote. Two of the 3R Su(var) loci have demonstrated lethality over Df(3R)*E-079*, one of which shows

semi-lethality in one recombinant strain, *B - Gl,A57*. If this semi-lethality is due to crossing off a second lethal, or even haplo-specific lethal, it may represent a locus such as Nash *et al.* (1983) suggest. Although this strain is not completely viable, survivors are fertile, suggesting that in this case, an extraneous lethal was involved in the lethal phenotype observed. This possibility should be of concern to investigators of dominant recessive lethal mutations.

Inter se complementation tests suggest that 2 or more complementation groups make up the 3L-3R proximal cluster (see Figure 12). Su(var)s *B143, A48, B94* and *C119* are sterile as trans-heterozygotes in all *inter se* combinations. Since Su(var)*B143* maps to 3L, all 4 alleles of this locus can be tentatively assigned to this location. Reuter *et al.* (1986) identified a locus, *Su-var(3)3*, which maps genetically and cytologically (via new compound formation) near the centromere on 3L. They report that mutants assigned to this locus are homozygous semi-lethal and sterile, with females producing no eggs. Males show a spread wing phenotype. This description matches the phenotypes observed in *B143* homozygotes exactly, strongly suggesting allelism to Reuter's *Su-var(3)3*.

Su(var) trans-heterozygotes in the complementation group observed in this study are completely female sterile with no eggs produced. They are also semi-lethal in males, which show a spread-wing phenotype. However, Su(var)*C119, A48* and *B94* do not show the same homozygous phenotypes as

FIGURE 12: Complementation groups based on trans-heterozygous phenotypes and physical mapping.



Su(var)*B143*.

When studying dominant mutations, it is always possible that trans-heterozygous phenotypes are a result of interactions between separate loci. In fact, this has been observed for Su(var)s in the 2L group (personal communication, Jo-Ann Brock). However, the observed behavior is best attributed to inter-allelic failure to complement for the following reasons. First, all four mutations in question have genetic map positions with overlapping 95% confidence levels, strongly suggesting one locus. Second, no interaction phenotypes were observed for any other Su(var)s tested, four of which are physically separated from this complementation group: Su(var)*A57* and *A63* by cytogenetic analysis and Su(var)*A130* and *A160* by genetic mapping. This question could be further addressed by mapping the Su(var) loci in question by compound-autosome formation. This could be done by capturing the newly formed compound with a compound strain that carries a *wm4* rearrangement (see appendix). A result showing 3L locations would strongly support the hypothesis that these Su(var) loci are allelic.

Of the remaining Su(var) mutants, only one is a non-essential locus, Su(var)*B76*. It is homozygous viable and fertile and shows no interactions with the clustered or non-clustered Su(var)s tested. Su(var)*B76* does, however, react to heterochromatin loss, just as the essential loci do, suggesting a similar function.

Locke *et al.* (1988) have hypothesized that all dominant modifiers of PEV are dosage sensitive and fall into one of two categories. Class I modifiers are haplo-insufficient, suppressing when hemizygous over a deficiency and enhancing when triploid for the locus. Class II modifiers are rare and have the opposite effects to dosage, suppressing when triploid and enhancing over a deficiency. They suggest the Su(var) mutations reported by Reuter *et al.* (1981) and Sinclair *et al.* (1983) are class I modifiers and therefore hypomorphic or amorphic loci. This possibility has not been disproven, since deficiency studies were uninformative in the most proximal 3R region. Neither is it possible to distinguish between the suppressors as haplo-insufficient loci or antimorphs. Both classifications fit the data presented in this thesis. Hypomorphic mutations are more plausible based on the dosage sensitivity reported by Reuter *et al.* (1987) and Locke *et al.* (1988).

Probable functions for these loci have been suggested by Sinclair *et al.* (1983) and others (Reuter *et al.* 1981, 1986; Locke *et al.* 1988). Based on phenotypes of suppression, recessive lethality, sterility and wing phenotypes, Su(var)s likely control chromatin condensation or contribute structurally to formation and or maintenance of heterochromatin. This hypothesized function is supported by recent work of James and Elgin (1986). They have isolated a non-histone chromosomal protein specific to heterochromatin. Through *in situ* hybridization, they have

mapped its cDNA to a locus very close to or the same as Su(var)*M43*, a suppressor locus isolated by Sinclair *et al.* (1983).

Genetic characterization of these mutants provides functional information and a solid background for molecular characterization. Using both genetic and molecular techniques, the exact role of these genes can be determined and ultimately lead to an understanding of gene regulation at the chromatin level.

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APPENDIX

A. LINEARITY CURVE

To determine if the microfluoremeter is reading pigment amounts in a linear fashion, the following protocol was used: Pigment from Oregon-R heads was extracted as described in Materials and Methods, Chapter 1. Dilutions were made to represent 1, 2, 3, 4 and 5 heads. For example, 2 ul supernatent + 8 ul 2-mercaptoethanol = 20% of Oregon-R pigment or the equivalent of 1 head (1/5=20%). Fluorescence of each sample was measured and resulting pigment levels are plotted in Figures A-1,2 and 3.

B. *LTX2*/SU(VAR)II DATA TO ACCOMPANY GRAPHS IN FIGURES 3A-F.

Pigment values are given in Tables Ba-f. Values are mean ± standard deviations. Asterisks indicate values statistically different from basal levels for each variegator tested, specific to each trial.

C. RECOMBINATION DATA FOR *SU(VAR)S A169* AND *A130*

See Figure C.

D. COMPOUND *WM4* STRAIN

Patroclinous males and virgin females were recovered from the compound autosome mapping study reported in Chapter 2 that made it possible to construct a compound 3 strain carrying *wm4*:

$$\underset{Y}{\underline{wm4};C(3L)ri;C(3R)e^{s}} \quad x \quad \underset{+}{\underline{wm4};C(3L)ri;C(3R)e^{s}}$$

$$\downarrow$$

$$wm4;C(3L)ri;C(3R)e^{s} \quad \text{progeny}$$

This is a valuable stock since the presence of a Su(var) can be detected in all viable new compound formations resulting from a screen such as the one used in this study.

FIGURE A: Oregon—R pigment value linearity assay.

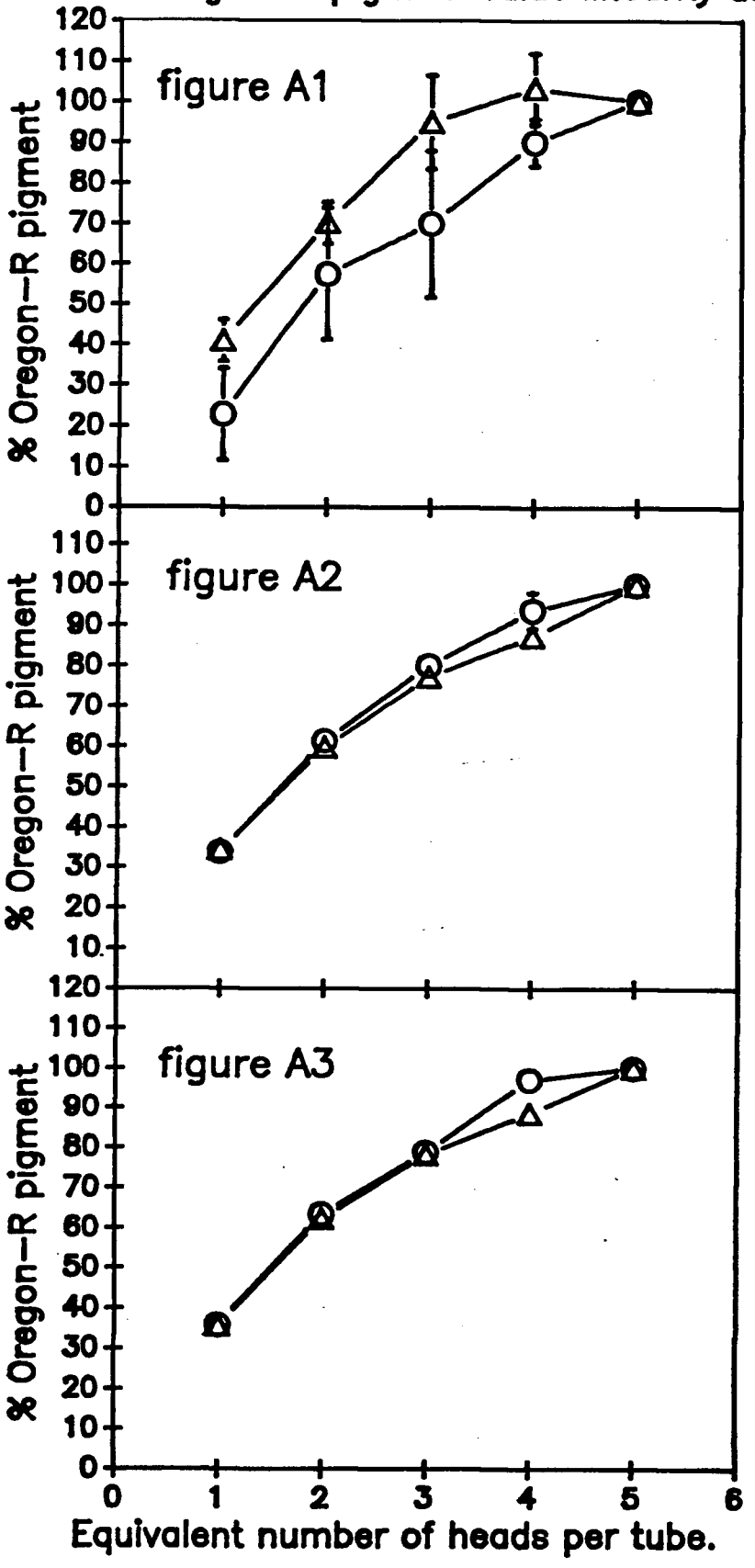

TABLE B.a: *LTX18*/SU(VAR) PIGMENT VALUES

SU(VAR)		TRIAL I	TRIAL II
T44	MALE	26.5 ± 1.4	*35.5 ± 2.9
	FEMALE	*21.0 ± 1.3	*34.0 ± 3.6
A151	MALE	21.7 ± 3.1	34.6 ± 1.3
	FEMALE	*20.6 ± 2.1	*33.5 ± 1.3
M43	MALE	*19.1 ± 2.1	27.3 ± 1.9
	FEMALE	*16.0 ± 1.4	*26.8 ± 1.3
B89	MALE	28.9 ± 0.6	N.D.
	FEMALE	29.2 ± 1.8	N.D.
A24	MALE	27.3 ± 2.2	*37.9 ± 3.9
	FEMALE	*24.5 ± 2.8	*40.6 ± 3.6
C157	MALE	*38.7 ± 1.2	*40.5 ± 4.8
	FEMALE	35.0 ± 4.4	*34.5 ± 1.3
H69	MALE	*22.1 ± 3.9	N.D.
	FEMALE	*24.7 ± 0.6	N.D.
M59	MALE	28.9 ± 4.9	*37.4 ± 2.9
	FEMALE	*22.5 ± 3.5	*32.9 ± 0.6

TABLE B.b: *LTX6*/SU(VAR) PIGMENT VALUES

SU(VAR)		TRIAL I	TRIAL II
T44	MALE	*33.0 ± 1.2	*30.5 ± 1.8
	FEMALE	*31.7 ± 0.5	*31.6 ± 2.0
A151	MALE	56.3 ± 6.3	*45.4 ± 1.4
	FEMALE	*35.9 ± 3.4	42.4 ± 2.2
M43	MALE	*29.8 ± 1.6	*34.3 ± 2.5
	FEMALE	*26.0 ± 0.7	*26.9 ± 1.6
B89	MALE	*48.4 ± 2.3	*40.8 ± 3.5
	FEMALE	*32.1 ± 1.8	*32.5 ± 1.7
A24	MALE	63.9 ± 3.5	52.1 ± 8.3
	FEMALE	*41.0 ± 2.2	43.1 ± 5.4
C157	MALE	*52.4 ± 8.2	56.9 ± 6.2
	FEMALE	*35.5 ±2.4	45.2 ± 5.4
H69	MALE	*51.3 ± 6.7	46.2 ± 10.5
	FEMALE	*32.7 ± 0.7	*37.1 ± 2.3
M59	MALE	*47.0 ± 0.9	48.1 ± 7.5
	FEMALE	*34.4 ± 2.6	*36.3 ± 3.2

TABLE B.c: *LTX2*/SU(VAR) PIGMENT VALUES

SU(VAR)		TRIAL I	TRIAL II
T44	MALE	*36.1 ± 6.3	*28.2 ± 1.4
	FEMALE	*36.5 ± 2.1	*31.8 ± 1.2
A151	MALE	*28.5 ± 1.7	*38.0 ± 3.2
	FEMALE	*35.0 ± 2.3	39.8 ± 4.2
M43	MALE	*32.5 ± 2.6	*25.8 ± 2.4
	FEMALE	*31.0 ± 1.2	*21.9 ± 0.8
B89	MALE	*29.1 ± 1.8	*34.1 ± 6.9
	FEMALE	*28.6 ± 1.4	*34.9 ± 3.7
A24	MALE	*41.7 ± 3.2	49.3 ± 6.2
	FEMALE	*37.0 ± 1.5	44.8 ± 2.4
C157	MALE	57.8 ± 4.2	50.6 ± 6.2
	FEMALE	*51.0 ± 2.2	41.9 ± 3.1
H69	MALE	*43.2 ± 3.0	*35.6 ± 4.7
	FEMALE	*43.5 ± 2.4	*28.6 ± 2.0
M59	MALE	*35.7 ± 2.6	N.D.
	FEMALE	*37.1 ± 2.5	*31.4 ± 1.4

TABLE B.d: *LTX13*/SU(VAR) PIGMENT VALUES

SU(VAR)		TRIAL I	TRIAL II
T44	MALE	*40.8 ± 2.4	*35.0 ± 0.8
	FEMALE	*32.4 ± 0.9	*39.8 ± 2.9
A151	MALE	84.8 ± 5.0	70.9 ± 5.9
	FEMALE	65.0 ± 0.8	68.5 ± 3.1
M43	MALE	*64.3 ± 3.6	*65.7 ± 7.2
	FEMALE	*47.3 ± 1.2	*53.0 ± 3.5
B89	MALE	84.5 ± 4.7	86.7 ± 4.9
	FEMALE	68.4 ± 3.0	*81.2 ± 4.0
A24	MALE	101.3 ± 2.1	94.6 ± 1.5
	FEMALE	*77.0 ± 3.4	*93.7 ± 2.3
C157	MALE	*74.9 ± 6.2	73.8 ± 9.0
	FEMALE	64.9 ± 4.7	72.6 ± 3.4
H69	MALE	*93.9 ± 3.0	91.8 ± 2.3
	FEMALE	67.6 ± 2.8	*82.5 ± 3.9
M59	MALE	80.7 ± 2.7	*67.0 ± 7.0
	FEMALE	68.6 ± 0.7	*61.7 ± 6.8

TABLE B.e: *LTX24*/SU(VAR) PIGMENT VALUES

SU(VAR)		TRIAL I	TRIAL II
T44	MALE	*54.3 ± 2.4	*36.7 ± 3.4
	FEMALE	*40.2 ± 2.3	*45.8 ± 2.4
A151	MALE	94.9 ± 7.8	*65.2 ± 5.5
	FEMALE	68.3 ± 5.8	*60.1 ± 4.7
M43	MALE	*77.9 ± 7.2	*67.0 ± 6.8
	FEMALE	*48.8 ± 5.1	*54.9 ± 6.7
B89	MALE	96.1 ± 4.1	85.1 ± 8.8
	FEMALE	66.7 ± 2.3	*71.9 ± 3.6
A24	MALE	113.4 ± 9.1	*97.6 ± 1.3
	FEMALE	81.6 ± 3.1	93.7 ± 2.3
C157	MALE	85.4 ± 1.9	N.D.
	FEMALE	71.7 ± 0.9	N.D.
H69	MALE	110.3 ± 4.4	96.1 ± 3.1
	FEMALE	69.5 ± 3.4	*79.5 ± 7.1
M59	MALE	83.9 ± 3.1	83.9 ± 2.9
	FEMALE	68.0 ± 3.1	72.8 ± 4.1

TABLE B.f: *LTX4*/SU(VAR) PIGMENT VALUES

SU(VAR)		TRIAL I	TRIAL II
T44	MALE	*71.5 ± 13.0	*35.8 ± 2.3
	FEMALE	*38.9 ± 1.2	*40.6 ± 3.0
A151	MALE	106.0 ± 4.5	*68.5 ± 4.9
	FEMALE	*79.5 ± 8.1	68.6 ± 4.8
M43	MALE	N.D.	*43.4 ± 4.9
	FEMALE	*59.7 ± 2.2	*49.8 ± 3.7
B89	MALE	99.7 ± 2.8	*83.2 ± 5.8
	FEMALE	82.7 ± 5.2	*72.2 ± 9.2
A24	MALE	117.8 ± 3.2	92.5 ± 1.4
	FEMALE	91.6 ± 5.1	88.7 ± 3.4
C157	MALE	*89.2 ± 2.4	83.1 ± 6.0
	FEMALE	*72.5 ± 1.5	75.6 ± 3.6
H69	MALE	109.6 ± 8.8	*83.5 ± 5.4
	FEMALE	81.0 ± 6.6	*80.3 ± 5.1
M59	MALE	*90.9 ± 6.8	*67.5 ± 6.2
	FEMALE	*76.4 ± 3.9	*64.0 ± 3.3

FIGURE C: RECOMBINATION DATA FOR *SU(VAR)S A160* AND *A130*

CROSS: *wm4* Su / *wm4 Gl Sb (H)* x *wm4* *Ly* / Y TM3 *e Ser*

OFFSPRING		*A130*	*A160*
PARENTAL:	*Gl Sb H* Su + +	270	916
SCO I:	Su *Gl Sb (H)* + + + (+)	39	401
SCO II:	+ *Gl* + (+) Su + *Sb (H)*	41	154
DCO:	+ + *Sb (H)* Su *Gl* + (+)	1	47
	TOTAL	351	1,518

order: Su--------*Gl*----------*Sb*-------*H*
I II

A130: <--11.4--><----12.0---->
A160: <--29.5--><----13.2---->

aproximate distances