Competition Between Different Variegating Rearrangements for Limited Heterochromatic Factors in *Drosophila melanogaster*

Vett K. Lloyd, Donald A. Sinclair and Thomas A. Grigliatti

Department of Zoology, University of British Columbia, Vancouver, British Columbia, V6T 1Z4 Canada

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ABSTRACT

Position effect variegation (PEV) results from the juxtaposition of a euchromatic gene to heterochromatin. In its new position the gene is inactivated in some cells and not in others. This mosaic expression is consistent with variability in the spread of heterochromatin from cell to cell. As many components of heterochromatin are likely to be produced in limited amounts, the spread of heterochromatin into a normally euchromatic region should be accompanied by a concomitant loss or redistribution of the protein components from other heterochromatic regions. We have shown that this is the case by simultaneously monitoring variegation of a euchromatic and a heterochromatic gene associated with a single chromosome rearrangement. Secondly, if several heterochromatic regions of the genome share limited components of heterochromatin, then some variegating rearrangements should compete for these components. We have examined this hypothesis by testing flies with combinations of two or more different variegating rearrangements. Of the nine combinations of pairs of variegating rearrangements we studied, seven showed nonreciprocal interactions. These results imply that many components of heterochromatin are both shared and present in limited amounts and that they can transfer between chromosomal sites. Consequently, even nonvariegation portions of the genome will be disrupted by re-allocation of heterochromatic proteins associated with PEV. These results have implications for models of PEV.

CHROMATIN structure has a profound and global effect on the expression of a gene. One of the most dramatic examples of the impact of chromatin structure on gene expression is the phenomenon of position-effect variegation (PEV). PEV is the random inactivation of a functional gene that has been repositioned into or next to a broken segment of heterochromatin (reviewed by LEWIS 1950, BAKER 1968 and SPOFFORD 1976). The resulting phenotype is a mosaic of wild type and mutant cells. The mosaic phenotype does not result from mutation in the gene itself (DUBININ and SIDOROV 1935; PANSHIN 1935, 1938; GRUNEBERG 1937; KAUFMANN 1942; HINTON and GOODSMITH 1950; JUDD 1955; TARTOF *et al.* 1984; LEVIS *et al.* 1985; REUTER *et al.* 1985) but instead is dependent on the proximity of the variegating gene to heterochromatin. A causal role for chromatin structure in PEV has been inferred from the correlation between genetic inactivation, in the tissue in which the gene is normally expressed, and the acquisition of a heterochromatic morphology in the corresponding genomic segment in the polytene chromosome. Moreover, the morphology of the polytene chromosome becomes more "euchromatic" or "heterochromatic" as the level of variegation is altered by various factors which modify PEV. These factors include temperature (HARTMANN-GOLDSTEIN 1967; ZHIMULEV *et al.* 1986), additional *Y*-chromosome heterochromatin (PROKOFYEVA-BELGOVSKAYA 1947; COWELL and HARTMANN-GOLDSTEIN 1980), other variegating rearrangements (HARTMANN-GOLDSTEIN and WARGENT 1975), deficiencies for the histone genes (KHESIN and LEIBOVITCH 1978; MOORE *et al.* 1983) and mutations that modify PEV (REUTER *et al.* 1982b; HAYASHI *et al.* 1990).

The mosaicism associated with PEV results from variable inhibition of the transcription of the variegating gene (BAHN 1971; NIX 1973; ANANIEV and GVOZDEV 1974; HENIKOFF 1981; RUSHLOW *et al.* 1984; KORNHER and KAUFFMAN 1986) and so presumably reflects the impact of altered chromatin structure on gene expression. The exact mechanism whereby "heterochromatinization" results in reduced gene expression remains unknown. Several different models for the molecular basis of PEV have been advanced (FRANKHAM 1988; KARPEN 1994; CSINK and HENIKOFF 1996) although alteration in chromatin structure remains among the most popular. Regardless of whether the alteration in chromatin structure is the cause or is simply associated with gene inactivation in PEV, the cytologically visible alteration in chromatin structure must at some level depend on specific protein components of heterochromatin. It seems plausible that these chromosomal proteins migrate across the newly formed boundary into normally euchromatic segments of the genome and impose a highly compacted state onto the genes in the affected region. This new structure presumably impedes access

Corresponding author: Thomas A. Grigliatti, Department of Zoology, University of British Columbia, 6270 University Blvd., Vancouver, British Columbia, V6T 1Z4 Canada. E-mail: grigliat@zoology.ubc.ca

by, or function of, the normal transcriptional machinery and thus, if not the sole cause of, at least assists in the inactivation of genes located in this region.

The strong correlation between the appearance of chromatin that morphologically resembles heterochromatin and gene inactivation has prompted the use of PEV to monitor the state of both the protein components of heterochromatin (SPOFFORD 1967; HENIKOFF 1979; REUTER and WOLFF 1981; REUTER *et al.* 1982b; SINCLAIR *et al.* 1983; GARZINO *et al.* 1992; DORN *et al.* 1993; BIRCHLER *et al.* 1994; CSINK *et al.* 1994) and the DNA sequences to which they bind (TARTOF *et al.* 1984; POKHOLKOVA *et al.* 1993). This approach has permitted the identification of genes encoding proteins associated with heterochromatin (JAMES and ELGIN 1986; EISSENBERG *et al.* 1990; REUTER *et al.* 1990). However, the number of chromatin-associated proteins is likely to be large, estimated from 20 (LOCKE *et al.* 1988) to 160 (SZIDONYA and REUTER 1988) and their binding sites are largely uncharacterized.

In the absence of a well-defined molecular system, two types of genetic approaches have been taken to study this complex system. In the first type of approach, the expression of a single variegating reporter gene is monitored while decreasing the amounts of different putative nonhistone chromosomal proteins, by using mutations that suppress PEV. The second approach relies on increasing the amount of DNA, which these chromatin proteins must package, typically by adding an extra *Y* chromosome, which is destined to be packaged as heterochromatin. Both of these approaches ultimately affect the stoichiometry of the components of heterochromatin by altering either levels of the wild-type heterochromatic proteins or the number of their binding sites. Although the ability of one variegating rearrangement to influence the variegation of another when combined has been noted (LEWIS 1950; BAKER 1968), this effect has not been well studied or exploited. Competition between two variegating rearrangements may allow the study of repatterning of the protein components of heterochromatin while preserving both the euploid genome content and wild-type levels of chromatin proteins.

The rational for this approach stems from the mosaic phenotype of PEV. For the sake of argument, let us assume that PEV is caused by, or associated with, a redistribution of chromatin proteins between different regions of a chromosome, namely from the heterochromatic region to the variegating euchromatic region. If these heterochromatic components are both limited in quantity and shared between different variegating rearrangements, then the deposition of heterochromatin-associated proteins at one site of the genome should reduce the availability of these proteins at another site. The dosage sensitivity of the histone gene region (KHESIN and LEIBOVITCH 1978; MOORE *et al.* 1983) and many suppressor and enhancer of PEV genes (LOCKE *et al.* 1988; WUSTMANN *et al.* 1989) suggest that at least some of the components for heterochromatin formation exist in limited supply. If these limited heterochromatin components are also shared between variegating rearrangements, combining different variegating rearrangements may promote competition for these components. This study is based on the prediction that variegating rearrangements themselves can yield information about the flux of heterochromatic proteins and their ultimate interaction to produce the different forms of chromatin structure associated with PEV.

Our results indicate that the inactivation of one set of euchromatic genes, by PEV, is accompanied by decreased expression of a heterochromatic gene. This result suggests a concomitant alteration in the integrity of portions of both the heterochromatin and the euchromatin within this one rearranged chromosome. We extended the possibility for competition between chromosomal regions by combining several different variegating rearrangements. Some pairwise combinations of variegating rearrangements showed nonreciprocal interactions whereas other combinations acted independently. These results suggest that at least some of the protein components of heterochromatin are (i) fairly labile, (ii) shared between different variegating rearrangements, and (iii) produced in limited amounts within the cell. Thus heterochromatic regions compete for this limited supply. As a result the presence of a variegating rearrangement may affect other variegating rearrangements as well as other parts of the genome that are not themselves variegating.

MATERIALS AND METHODS

Mutant strains and chromosomes: The mutations and rearranged variegating chromosomes used in this study are described in LINDSLEY and ZIMM (1992). The *X*-linked *white* variegating rearrangements, $In(1)w^{m4}$, $In(1)w^{mMc}$, $In(1)w^{m51b}$ and $T(1;4)w^{mJ}$, will be hereafter referred to as w^{m4}, w^{mMc}, w^{m51b}, and w^{mJ}, respectively. The autosomal rearrangements $T(2;3)Sb^{V}$, $In(2R)bw^{VDr2}$, and $T(2;3)lt^{rx13}$ will be hereafter referred to as Sb^{V}, bw^{V}, and lt^{rx13}, respectively.

Crosses: All crosses were performed at 22° unless otherwise stated. Flies were grown on standard cornmeal/sucrose media supplemented with antibiotics and 0.04% tegosept as a mold inhibitor. Crosses generally involved five groups of three to five virgin females crossed to an equal number of males in 8-dram shell vials. The crosses were subcultured twice at 4–5 day intervals before the parents were discarded. Each set of crosses was scored independently. The data from replicate crosses within a group were subsequently pooled, since there were no differences between replicates.

Simultaneous variegation for heterochromatic and euchromatic variegators: A number of chromosomes variegating for the heterochromatic gene *light* (*lt*) were kindly provided by Dr. B. WAKIMOTO. These chromosomes were tested in heterozygous combinations with recessive mutations located near the euchromatic breakpoint of the rearranged chromosome as follows: lt^{+} females from the different *light* variegated strains were crossed with males carrying recessive point mutations in genes adjacent to the lt^{+} breakpoint. The progeny were scored for appearance of the recessive phenotype in heterozygous

flies. An individual was scored as mutant for *raised* if it displayed a raised-wing phenotype after three successive trials (that is, after being disturbed by knocking the fly to the bottom of the vial). A fly was classified as mutant for *brief* if it was one half or less of the size of its siblings, mutant for *crumpled* if the wings were collapsed, and mutant for *white occelli* if the occelli were unpigmented. In each case, the mutant phenotype in the *light* variegator/mutant individual was less extreme and more variable than in the homozygous mutant stock. The mutant phenotypes were scored very conservatively and biased against variegation. Furthermore, the incidence of these mutant phenotypes was adjusted for false positives by subtracting the percent of abnormal phenotypes observed in the $lt^{vX13}/TM3$ siblings. Female and male data were combined as no differences were noted.

Interactions between different variegators: In order to minimize genetic background effects, due to possible preexisting modifiers of PEV, strains were constructed in which the first, second and third chromosome were all derived from the same marked strain. Four strains were constructed, each with a different variegating *white* allele on an inverted *X* chromosome (either w^{m4} or w^{mMc} or w^{m51b} or w^{mf}) and with the second chromosome inversion *brown* variegated (bw^V) combined with the *Stubble* variegated (Sb^V) translocation involving the second and third chromosomes.

***bw*^{V} and *Sb*^{V} interactions:** Double and single variegator-bearing flies were generated in the first instance by crossing bw^V/CyO females or males to Sb^V/CyO flies of the appropriate sex. No parental effects were noted. More extensive analysis of bw^V and Sb^V interactions were performed on double and single variegator bearing flies derived from crossing w^{m4}/Y; bw^V/Sb^V, w^{mMc}/Y; bw^V/Sb^V, w^{m51b}/Y; bw^V/Sb^V and w^{mf}/Y; bw^V/Sb^V males to +/+; bw^V/Sb^V females. The double-variegator bearing flies were assayed for variegation of both *brown* and *Stubble*. Some of the F_1 males were crossed to wild-type (Canton S) females to generate single-variegator bearing individuals that were also assayed for either *brown* or *Stubble* variegation.

***white* and *Stubble* or *brown* interactions:** Males carrying three variegators, *white, brown* and *Stubble* (*e.g.*, w^m/Y; bw^V/Sb^V) were crossed to females homozygous for the corresponding *white mottled* (*e.g.*, w^m/w^m; +/+) chromosome but otherwise wild type. The progeny were assayed for *white* bw^V and Sb^V variegation. Some of the male progeny from this cross were backcrossed to w^m/w^m; +/+ females. Progeny were again assayed for levels of *white* and bw^V or *white* and Sb^V variegation. Siblings bearing only the *white* variegator were used as internal controls. Progeny carrying two variegators ($w^m/+$; $bw^V/+$ or $Sb^V/+$) were crossed to *Canton S* females to generate offspring either wild type or heterozygous for the recessive variegators, which served as internal controls. Finally, males carrying either the bw^V or Sb^V chromosome as their only variegating chromosome were again crossed to wild-type (*Canton S*) females to generate both wild-type flies and females with one variegating chromosome, either $bw^V/+$ or $Sb^V/+$.

Localizing the suppressing ability of the *w*^{mMc} chromosome: Three approaches were taken to ensure that the interactions observed between different combinations of variegators were due to interactions between the variegating portions of the chromosomes, rather than general effects of genetic background. The ability of the w^{mMc} chromosome to suppress Sb^V variegation was used for these studies since it was the most extreme interaction.

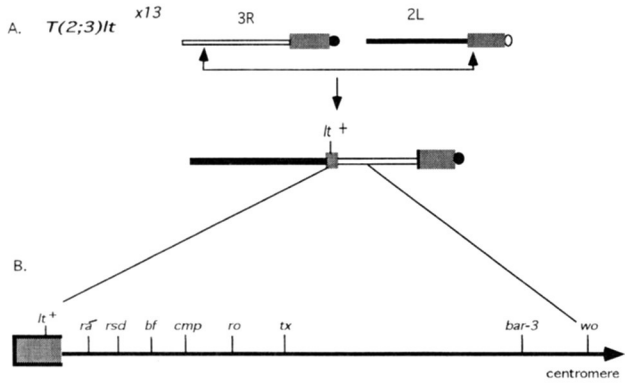
First, the w^{mMc} chromosome was reisolated after extensive outcrossing to a unrelated, nonvariegating $white^-$ strain that had never encountered a variegating chromosome. This $white^-$ strain had no effect on *Stubble* variegation. For these crosses, w^{mMc}/w^{mMc} females were crossed to w^-/Y males. The w^{mMc}/w^- females from this cross were collected and crossed to nonsibling w^-/Y males. After 17 successive generations of outcrossing heterozygous w^{mMc} females were crossed with +/*Y*; $Sb^V/SM1$ males to determine their effect on *Stubble* variegation. Second, the suppressing ability of the w^{mMc} chromosome was mapped by recombination as follows. *y cv v f* females were crossed with w^{mMc}/Y males. The F_1 were allowed to mate *inter se*. Individual F_2 males bearing recombinant *X* chromosomes were isolated and crossed to *C(1)DX, yf/Y* females to establish a stock. Males from these stocks were then crossed to +/+; $Sb^V/SM1$ females and their progeny were assayed for their effect on Stubble variegation. Third, the suppressing ability of the w^{mMc} chromosome was further localized to the proximal or distal variegating junction by recombinationally separating the disrupted boundary regions. As the breakpoints of the nonsuppressing w^{m4} and the suppressing w^{mMc} rearrangements are close, a single crossover between them will exchange homologous regions of the chromosomes. This generates chromosomes with either the w^{mMc} distal junction coupled to the w^{m4} proximal junction or the converse. Males bearing w^{mMc} marked with a combination of the *y cv v f* markers from the previous recombination experiment were crossed to w^{m4}/w^{m4} females. The F_1 were allowed to mate and males bearing recombinant distal w^{mMc} and proximal w^{m4} boundary chromosomes, or the converse, were selected. These males were then crossed to +/+; $Sb^V/SM1$ females to determine the effect of the distal and proximal w^{mMc} regions on *Stubble* variegation.

Assays to quantify variegation: *white and brown variegation:* The amount of pigment deposited in the eye was measured separately for 25 females and 25 males. Flies 3 to 7 days posteclosion were decapitated by vigorously banging the frozen flies in an empty screw cap tube. The heads were placed in wells of a microtiter plate and 30 μl of 0.25 M β-mercaptoethanol in 1% aqueous NH_4OH was added to each well. The eye pigment was released by sonication for 3 sec and a 5-μl aliquot was removed from each well and applied to a piece of Whatman no. 3 filter paper. The amount of pigment in the dried spot was determined fluorometrically using a MPS-1 Ziess microscope. A minimum of five groups, with five heads per group, were measured for each genotype and sex. In each case, the amount of pigment in each of the five spots was averaged and expressed relative to wild type.

Quantification of Stubble variegation: Fourteen major bristles, (posterior supra-alars, anterior post-alars, posterior dorsocentrals, and anterior and posterior scutellars and sternopleurals on each side of the fly) were examined and assigned either a mutant *Stubble* or a wild-type phenotype. This value was expressed as a percentage of the fully mutant phenotype since the *Stubble* variegating rearrangement variegates for the expression of the dominant *Sb* mutation.

RESULTS

Simultaneous variegation of heterochromatic and euchromatic genes: The phenotype of PEV, in itself, suggests a reassignment of protein components from the normally heterochromatic portion of the genome to the transposed and now variegating euchromatic segment. However, in most previous studies on PEV, only one region of the genome has been monitored for variegation, usually only the "gene rich" euchromatic junction. Therefore, the redistribution of heterochromatin constituents can only be inferred. There are, however, a few rearrangements that show variegation of heterochromatic genes, by virtue of break points close to these genes. To reveal redistribution of hetero-



C.

percent lt^{vx13} /mutant showing variegation for the euchromatic gene

ra	rsd	bf	cmp	ro	tx	bar-3	wo
0	3	8	4	0	0	0	1

FIGURE 1.—Simultaneous variegation for euchromatic and heterochromatic genes. (A) Schematic representation of *T(2;3)lt*^{x13} chromosome. The normally heterochromatic wild-type gene for *light (lt)* is translocated to distal 2L. The open line represents 3R euchromatin; the stipple line represents euchromatin of the second chromosome and the striped box represents heterochromatin. (B) Expanded diagram of the euchromatic region of 2L abutting the translocated heterochromatic segment. The genes tested for variegation are shown above the line. (C) Percent of individuals showing expression of the mutant phenotype of each tested euchromatic gene when the *lt*^{x13} translocation is heterozygous with a recessive mutant allele of each euchromatic gene. Values are average of male and female data at 22°.

chromatin components between different parts of the chromosome, we have monitored the expression of genes located within the heterochromatin, at one side of the breakpoint, as well as genes located within the juxtaposed segment of euchromatin.

A number of rearrangements that variegate for the heterochromatic gene *light* were tested for variegation of euchromatic genes adjacent to both the proximal and distal breakpoints (*rough, crumpled, brief, raised and rasé, white occelli, bar-3,* and *taxi*). One of these chromosomes (lt^{vx13}) showed mutant phenotypes when heterozygous with recessive alleles of four euchromatic genes: *raised, brief, crumpled* and *white occelli* (Figure 1). As previously reported (HESSLER 1958; WAKIMOTO and HEARN 1990), the light-eye mutant phenotype in these individuals was intensified by the addition of extra heterochromatin to the genome and suppressed by the removal of heterochromatin from the genome. The amount of pigment, measured by microflourimetry, in lt/lt^{vx13} euploid females and males was 60 and 77% of wild-type levels, respectively. This amount decreased to 42% in *XXY* females and increased to 85% in *XO* males. The distance of spread of inactivation into the euchromatic region (assessed by the fraction of mutant individuals) is increased by low temperature and by addition of a *Y* chromosome, as expected (data not shown). The low penetrance, variable hypomorphic phenotypes, temperature sensitivity and sensitivity to relative amount of heterochromatin in the genome of these mutant phenotypes is indicative of PEV and suggests that these phenotypes arise as a result of cis-inactivation of the wild-type copy of the euchromatic gene juxtaposed to the heterochromatic breakpoint.

Transfer of some chromatin proteins from heterochromatin to the juxtaposed, normally euchromatic segment provides a simple hypothesis for the coupling of heterochromatic and euchromatic variegation in this single rearrangement. This redistribution or "bleeding" of components of heterochromatin to the normally euchromatic segment of DNA could cause the variegation of the euchromatic genes, while the concomitant loss of heterochromatin from the normally heterochromatic segment results in reduced expression of the *light* gene. In the lt^{vx13} chromosome, the lt^{+} gene and a large block of its associated heterochromatin is moved to the terminal euchromatic portion of chromosome *3* (WAKIMOTO and HEARN 1990), and as a result, both regions experiencing variegation are contiguous. Thus an explanation for the coincident variegation of both the normally euchromatic and heterochromatic loci might be provided by postulating a physical relocation of the variegating chromosomal segment from one nuclear compartment to another. This physical relocation to a different nuclear compartment may lead to alternate packaging and repression of the lt^{+} and euchromatic genes, or alternatively, as postulated by TALBERT *et al.* (1994), it may sequester the reporter genes into a compartment lacking the one or more transcription factors required for their expression. To extend the possibilities for interactions between variegating regions, we constructed strains with one, two, three, or four different unlinked variegating rearrangements.

Interactions between different variegating systems: competition for limited heterochromatic factors: Our findings that protein redistribution can occur between different sites within one chromosome, suggests that competition might also occur between sites on different chromosomes if they share one or more protein components of heterochromatin and if these shared heterochromatic components exist in limited quantities. To test for competition between different variegating regions, two (or more) different, unlinked variegation rearrangements were combined within the same individual. Theoretically, two different variegating rearrangements might affect each other in one of three ways:

1. They might show a reciprocal effect: suppression of the variegation of one rearrangement coupled with enhancement at the other. This result suggests a transfer of heterochromatic components from one

variegating junction to another, presumably without the involvement of other regions of the genome.

2. The expression of the variegating gene, or genes, in one rearrangement might be suppressed or enhanced while variegation at the second remained unaffected. This situation indicates redistribution of chromatin proteins within the whole genome. A net loss (suppression) or addition (enhancement) of heterochromatic components at one variegating euchromatic region is coupled with the ability of nonvariegating regions of the genome to donate or absorb these heterochromatic components. On the other hand, the physical consignment of one variegating rearrangement to an alternate compartment within the nucleus may exclude the second variegating rearrangement from occupying the same compartment. Thus the rearrangement, occupying the distinct compartment, would variegate as it usually does, while the second rearrangement, by virtue of its exclusion from this compartment, may occupy its normal compartment and thus appear suppressed.
3. The two variegating segments may behave independently. Variegation at one region might have no effect on the level of variegation of the other. In the chromatin packaging model for PEV, failure to compete might be due to the two variegating rearrangements having no sequence or protein components in common, an effectively unlimited supply of the shared components, or to the inability of the protein components to transfer between different chromosomes. In the nuclear compartment model for PEV, failure to compete may indicate that both variegating rearrangements can occupy a single compartment or that each may occupy two distinct compartments, each of which is unable to support expression of the variegating loci.

To determine if the protein components of heterochromatin can be reapportioned between different variegating rearrangements or if two rearrangements compete for a single compartment, strains with one, two, three or four different variegating rearrangements were constructed. The degree of variegation occurring in individuals with two, or more, variegating rearrangements was compared to siblings bearing only one type of each variegator to determine if the variegating rearrangements interacted.

Interactions between *brown* and *Stubble* variegating chromosomes: Both Sb^V and bw^V are dominant autosomal variegators. Since they affect different tissues (bristle morphology and eye pigmentation, respectively) they can be monitored independently. In addition, monitoring genes expressed in different tissues *vs.* two genes expressed in the same tissue (see interactions between *white*-mottled and *brown* variegators below) should allow us to monitor the effect of transcription on competition. Strains were constructed with both

TABLE 1

Interactions between *brown* and *Stubble* variegated

	bw variegation[a]		*Sb* variegation	
	bw^V alone[b]	bw^V and Sb^{Vd}	Sb^V alone[c]	Sb^V and bw^{Vd}
♀	31 ± 2	49 ± 5	63 ± 3	53 ± 4
♂	45 ± 4	64 ± 5	57 ± 4	59 ± 5

Cross:

w^{m-}; $Sb^V/bw^V \otimes +/+$; Sb^V/bw^V

↓

$+/Y$; $Sb^V/bw^V \otimes +/+$; $+/+$ (bw^V and Sb^V)

↓

$+/Y$; $Sb^V/+$ or $bw^V/+$ and $+/Y$; $Sb^V/+$ or $bw^V/+$ (bw^V or Sb^V alone),

where *a* is all values are expressed as percent full gene expression; *b* is brown alone = $+/+$; $bw^V/+$ or $+/Y$; $bw^V/+$; *c* is Stubble alone = $+/+$; $Sb^V/+$ or $+/Y$; $Sb^V/+$; and *d* is brown and Stubble = $+/+$; bw^V/Sb^V or $+/Y$; bw^V/Sb^V.

brown and *Stubble* variegated rearrangements on the autosomes and then out crossed to yield double- and single-variegating flies. Expression of bw^V and Sb^V in the double-variegating flies was compared to siblings bearing only one of these variegators (Table 1). The presence of the Sb^V rearrangement consistently suppressed the variegation of the *brown* gene. Interestingly, however, the presence of the *brown* variegator had little or no effect on *Stubble* variegation.

Interactions between *white* mottled and *Stubble* variegated chromosomes: We tested the interactions between four different *white* variegators (w^{m4}, w^{mMc}, w^{m51b}, and w^{mJ}) and *Stubble* variegated. Once again, these two variegating genes affect different tissues, allowing independent determination of the extent of variegation of each locus. Since the *white* variegators are viable as homo-, hemi- and heterozygotes, it was also possible to determine whether variegation was sensitive to dose of the *white* mottled rearrangement (*e.g.*, if the interaction is dominant or recessive). Finally, the use of four different rearrangements that variegate for the same euchromatic gene (hence have similar euchromatic breakpoints) but have different heterochromatic breakpoints allowed us to determine whether the competition was dependent on the euchromatic region or the heterochromatic segment to which the *white* gene was juxtaposed.

Table 2 shows the results of these crosses. The effect of two of the *white* variegators on Sb^V was dramatic. The presence of the w^{mMc} chromosome strongly suppressed the variegation of the *Stubble* gene in both females and males (from approximately 60% expression in the absence of w^{mMc} to 100% expression in its presence). Furthermore, this effect was associated exclusively with the w^{mMc} breakpoint (see below). A single w^{mMc} chromosome was sufficient to suppress Sb^V even in the presence of a

TABLE 2

Interactions between *Stubble* and *white* variegators

	white variegation[a]		*Stubble variegation*[a]		
	white alone[b]	white and Stubble[c]	Stubble alone[d]	Stubble and white (one copy)[e]	Stubble and white (two copies)
w^{m4}					
♀	14 ± 4	24 ± 4	59 ± 3	66 ± 5	79 ± 4
♂	9 ± 4	23 ± 5	49 ± 4	66 ± 5	
w^{mMc}					
♀	100 ± 5	88 ± 6	61 ± 3	91 ± 2	97 ± 2
♂	52 ± 7	79 ± 5	54 ± 4	98 ± 2	
w^{m51b}					
♀	99 ± 1	91 ± 1	63 ± 3	72 ± 4	90 ± 4
♂	81 ± 8	87 ± 5	62 ± 4	87 ± 4	
w^{mJ}					
♀	80 ± 10	72 ± 5	46 ± 4	62 ± 3	49 ± 3
♂	86 ± 5	88 ± 5	58 ± 5	45 ± 3	

Crosses:

$$w^{m-}/Y;\ Sb^{V}/bw^{V} \otimes w^{m-}/w^{m-};\ +/+$$

$$\downarrow$$

$$w^{m-}/Y;\ Sb^{V}/+^{d} \otimes w^{m-}/w^{m-};\ +/+$$

$$\downarrow$$

$$w^{m-}/w^{m-};\ +/+^{b} \text{ and } w^{m-}/Y;\ +/+^{b} \text{ and } w^{m-}/w^{m-};\ Sb^{V}/+^{e} \text{ and } w^{m-}/Y;\ Sb^{V}/+^{d} \otimes +/+;\ +/+$$

$$\downarrow$$

$$w^{m-}/+;\ Sb^{V}/+^{d} \text{ and } +/Y;\ Sb^{V}/+^{c} \otimes +/+;\ +/+$$

$$\downarrow$$

$$+/+;\ Sb^{V}/+^{c} \text{ and } +/Y;\ Sb^{V}/+^{c},$$

where *a* is all values are expressed as percent full gene expression ± standard error of the mean; *b* is white variegated alone = w^{m}/w^{m}; +/+ or w^{m}/Y; +/+; *c* is Stubble variegated alone; *d* is Stubble variegated with one copy of white variegated; and *e* is Stubble variegated with two white variegated alleles.

wild-type chromosome (compare Sb^{V} alone with Sb^{V} and one copy of w^{mMc}). However, two copies of the w^{mMc} produced a slightly greater suppression than one copy. Thus, this interaction is both dominant and dosage sensitive. The w^{m51b} and w^{m4} chromosomes had similar, but less dramatic, suppressing effects on Sb^{V} expression. In contrast, the w^{mJ} chromosome had no clear effect on the expression of the Sb^{-} allele in Sb^{V} chromosome. These results also show that the variegating euchromatic gene (*white*) is neither the cause, nor does it influence the magnitude, of the interaction since this gene was the same in all four *white* variegating rearrangements.

The Sb^{V} chromosome does not have a dramatic, reciprocal effect on any of the *white* variegators. The Sb^{V} chromosome may have a slight suppressing effect on w^{m4} and w^{mMc} males, and a suppressing effect might escape notice in both w^{mMc} and w^{m51b} since both appear to be weak variegators, but with the exception of w^{mMc} males, the effect, if any, is very weak. Clearly, the Sb^{V} chromosome does not cause a reciprocal enhancement of any of the *white* variegators. In summary, the results in Table 2 demonstrate that while the Sb^{V} chromosome has no dramatic effect on the *white* variegators at least some of these *white* variegators can strongly suppress the variegation of Sb^{V}. Thus, these are very clear cases of nonreciprocal interactions.

Interactions between the *white* mottled and *brown* variegated chromosomes: We next tested for interactions between the *white* variegators and bw^{V}. Both these genes are expressed in the eye pigment cells. Therefore, it was not possible to monitor their effects separately and consequently interactions cannot be assigned unequivocally to one variegating rearrangement or the other. However, we can measure the interactions between the two variegators. Since the bw^{V} rearrangement alone reduces the amount of red eye pigment to ~30–35% of wild-type levels and since the effects of the four different *white* variegators, acting alone, on pigment deposition have been determined (*white* alone column of Table 3), then the assumption that they act independently predicts that the resultant level of pigmentation will simply be the combination of these effects, *i.e.*, 30% of the pigment level of each *white* variegator alone. Devia-

TABLE 3

Interactions between *brown* and *white* variegators

	white alone[b]	brown alone[c]	brown and white[d]		brown and white[e]	
			Exp.	Obs.	Exp.	Obs.
w^{m4}						
♀	18	33	33	ND	6	4 ± 1
♂	13	36	5	10 ± 2		
w^{mMc}						
♀	99	33	33	87 ± 6	33	80 ± 8
♂	52	36	27	48 ± 5		
w^{m51b}						
♀	99	33	33	68 ± 4	33	56 ± 6
♂	52	36	27	54 ± 4		
w^{mJ}						
♀	83	33	33	6 ± 1	27	5 ± 1
♂	40	36	14	5 ± 1		

Crosses:

$$w^{m-}/Y;\ Sb^V/bw^V \otimes w^{m-}/w^{m-};\ +/+$$

$$\downarrow$$

$$w^{m-}/Y;\ bw^V/+^d \otimes w^{m-}/w^{m-};\ +/+$$

$$\downarrow$$

$$w^{m-}/w^{m-};\ +/+^b \text{ and } w^{m-}/Y;\ +/+^b \text{ and } w^{m-}/w^{m-};\ bw^V/+^e \text{ and } w^{m-}/Y;\ bw^V/+^d \otimes +/+;\ +/+$$

$$\downarrow$$

$$w^{m-}/+;\ bw^V/+^d \text{ and } +/Y;\ bw^V/+^c \otimes +/+;\ +/+$$

$$\downarrow$$

$$+/+;\ bw^V/+^c \text{ and } +/Y;\ bw^V/+^c,$$

where *a* is all values are expressed as percent full gene expression ± standard error of the mean; *b* is white variegated alone = w^m/w^m; +/+ or w^m/Y; +/+; *c* is brown variegated alone = +/+; $bw^V/+$ or +/*Y*; $bw^V/+$; *d* is brown variegated with one copy of white variegated; and *e* is brown variegated with two white variegated alleles. Exp., expected pigment values if variegators act independently; Obs., observed pigment values; ND, not done.

tions from this value indicate an interaction between the two variegating rearrangements.

The combinations of w^{mMc} with bw^V and w^{m51b} with bw^V have eye pigment levels much greater than expected (Table 3), indicating suppression of variegation of one or both eye color genes. In contrast, w^{mJ} when combined with bw^V shows a distinct reduction in eye pigment levels, indicating enhancement of the variegation of one or both genes. Finally, the w^{m4} and bw^V combination produced eye pigment levels in accord with the hypothesis of independent action. However, in this last case, we cannot exclude the possibility that one variegator was suppressed and the other enhanced to produce no net effect.

Linkage analysis showed that the ability to cause suppression, in the case of w^{mMc} and w^{m51b}, and enhancement, in the case of w^{mJ}, segregated with the *white* variegated chromosome (data not shown). Interestingly, the suppression by the w^{mMc} and w^{m51b} chromosomes were again dominant. The persistence of the suppression even in the presence of a wild-type *X* chromosome, which masks expression of the *white* variegator, implies that the variegation of the bw^V chromosome is suppressed and this suppression is induced by the w^m chromosome rather than the converse.

Simultaneous interactions between three and four variegating chromosomes: Having defined the interactions between pairs of variegating rearrangements, it seemed possible to maximize the potential for interactions and to define a hierarchy of competition by simultaneously combining three and four variegating rearrangements. It is possible that results of these experiments are less easily interpreted, since multiple interactions may be occurring. Fortunately, these data are all consistent with the results of competitions between individual pairs of variegators (Table 4). The w^{m4} chromosome, when combined with both Sb^V and bw^V, continues to show no pronounced suppression of Sb^V. The effect on eye pigmentation can be attributed to the suppressing effect of Sb^V on bw^V. The w^{mMc} rearrangement, which strongly suppresses Sb^V in pairwise combinations, continues to do so in the presence of bw^V. The suppressing effect of w^{mMc} on Sb^V is as strong in the presence of bw^V as it is in its absence (99 *vs.* 97%, respec-

TABLE 4

Interactions between three and four variegators

Genotype	No. of variegators	Eye pigment	Stubble variegation
w^{m4}/w^{m4}; Sb^V/bw^V ♀	4	8 ± 3	78 ± 3
w^{m4}/Y; Sb^V/bw^V ♂	3	8 ± 3	70 ± 4
$w^{m4}/+$; Sb^V/bw^V ♀	3	69 ± 3	69 ± 4
w^{mMc}/w^{mMc}; Sb^V/bw^V ♀	4	83 ± 2	99 ± 1
w^{mMc}/Y; Sb^V/bw^V ♂	3	56 ± 3	95 ± 2
$w^{mMc}/+$; Sb^V/bw^V ♀	3	86 ± 5	76 ± 4
w^{m51b}/w^{m51b}; Sb^V/bw^V ♀	4	66 ± 2	85 ± 3
w^{m51b}/Y; Sb^V/bw^V ♂	3	61 ± 3	84 ± 3
$w^{m51b}/+$; Sb^V/bw^V ♀	3	67 ± 4	62 ± 4
w^{mJ}/w^{mJ}; Sb^V/bw^V ♀	4	55 ± 3	79 ± 3
w^{mJ}/Y; Sb^V/bw^V ♂	3	52 ± 3	83 ± 4
$w^{mJ}/+$; Sb^V/bw^V ♀	3	66 ± 4	59 ± 2

All values are expressed as percent full gene expression ± SEM.

tively) and is again dosage sensitive. The eye pigment levels are also elevated in these flies, suggesting that the suppression of bw^V by w^{mMc} is unabated by the presence of Sb^V (83% with w^{mMc}, bw^V and Sb^V compared to 80% without Sb^V). The effect on eye pigmentation could be due to the suppressing ability of either the *white* variegator or Sb^V as both suppress bw^V. The interactions between one (males) or two (females) copies of w^{m51b} with bw^V and Sb^V show a similar pattern. Again, the *Stubble* variegating chromosome is suppressed regardless of the presence of *brown* variegation (85 *vs.* 90% with and without bw^V, respectively, for females, and 84 *vs.* 87% with and without bw^V in males). Suppression of eye pigmentation is also evident in these triple and quadruple variegating flies (w^{m51b}/Y or w^{m51b}/w^{m51b}; Sb^V/bw^V), although in this case the presence of Sb^V appears to reduce the suppression of bw^V and/or w^{m51b} relative to the suppression seen with pairwise combinations (66% with Sb^V *vs.* 91% without Sb^V in females, and 61% *vs.* 87% with and without Sb^V in males). Finally, combinations between w^{mJ}, bw^V and Sb^V show a slight suppressing effect on Sb^V. This effect is unexpected, since neither bw^V nor w^{mJ} suppress Sb^V expression in pairwise combinations. In addition, there is a rather dramatic suppression of variegation of eye pigment genes in this triple-variegator combination (55% in females and 52% in males) relative to the bw^V and w^{mJ} combination (6% in females and 5% in males). This suppression of gene inactivation (resulting from PEV) is comparable to the suppressing effect of Sb^V on bw^V, and thus it seems that the suppression of bw^V by Sb^V might take precedence over, or be epistatic to, the enhancement of bw^V by w^{mJ}.

In summary, a number of patterns of interactions emerge. First, it appears that different variegation rearrangements do not necessarily act independently, and in fact, most do not. One variegating rearrangement may either suppress (such as the effect of Sb^V on bw^V or of w^{mMc} and w^{m51b} on both Sb^V and bw^V) or enhance (w^{mJ} on bw^V) the variegation of another. The interactions appear not to be reciprocal. Second, the pattern of the interactions between different combinations of variegation chromosomes appears to be quite consistent. For example, the w^{mMc} and w^{m51b} chromosomes suppresses both Sb^V and bw^V. This result suggests that transcription is not required for competition to occur, while the *white* and *brown* genes are both expressed in the eye pigment cells, the *Stubble* gene is not and there is no evidence that the w^+ or the bw^+ gene is expressed in the bristle cells. That competition occurs in the absence of a requirement for gene transcription may indicate that the variegating chromosome domains are packaged or consigned similarly in all cells of the body, regardless of their transcriptional fate. Third, the w^{m4} rearrangement seems to act independently of all the other variegators suggesting that there are circumstances where competition need not occur. Assuming that PEV is associated with altered chromatin packaging, the observation that interactions occur indicates that the amount of at least some chromatin proteins is limited and that when the demand for these proteins in chromatin assembly is altered, there is a redistribution of heterochromatic protein components between variegating chromosomes. The nonreciprocal nature of the interactions indicates that reapportionment of heterochromatic proteins involves more than a simple loss of components (suppression) at one variegating locus with equivalent addition of these proteins at the other variegating locus (enhancement). Thus the heterochromatic protein components that are no longer associated with the suppressed heterochromatic/euchromatic interface must be engaged elsewhere in the genome.

Localizing the suppressing ability of the w^{mMc} chromosome to the proximal variegating junction: The ability of certain combinations of variegating chromosomes to interact could be due to either a genuine exchange of heterochromatic proteins from one variegating break-

point to another, or the presence of genetic modifiers of position-effect present on these chromosomes. The latter possibility was a concern because many established stocks containing variegating rearrangements have acquired extraneous modifying mutations (SPOFFORD 1967; SINCLAIR *et al.* 1989, and V. LLOYD unpublished results). These spontaneous allele configurations are presumably selected as they reduce inactivation of variegating euchromatic genes many of which are likely to have an effect on viability or fertility. In the experiments presented here, presence of modifying loci on the autosomes can be excluded since these chromosomes were replaced while constructing the multiply variegating stocks. Additionally, segregation studies of the suppression of both Sb^V and bw^V by the w^{mMc} and w^{m51b} chromosomes showed that this suppression was linked to the variegating *X* chromosome. It is formally possible that *X*-linked dominant modifier mutations on the w^{mMc} and w^{m51b} chromosomes were responsible for suppression of Sb^V and bw^V rather than competition between the two variegating regions for limited materials. In order to resolve this issue, we mapped, by recombination, the suppressing ability of the chromosome which caused the most extreme suppression, that of the w^{mMc} chromosome on Sb^V.

Two lines of evidence suggest that the ability of the w^{mMc} chromosome to suppress Sb^V is a property of the variegating euchromatic/heterochromatic boundaries rather than an unrelated suppressor mutation present elsewhere on the chromosome. In the first study, a w^{mMc} chromosome was outcrossed to a nonvariegating strain (which had no effect on Sb^V). After 17 generations of outcrossing, the original autosomes should have been replaced by segregation and much of the *X* chromosome by recombination. The extensively out-crossed w^{mMc} chromosome was indistinguishable from the original w^{mMc} chromosome in its ability to suppress Sb^V. Sb^V expression was 96 ± 1% with the out-crossed w^{mMc} chromosome *vs.* 91 ± 2% with the original w^{mMc} chromosome. In a second study, recombinants between the nonsuppressing, multiply marked chromosome *y cv v f* and the suppressing w^{mMc} chromosome were generated (Figure 2A) and tested for their effect on Sb^V. Figure 2B shows the effect of the two parental chromosomes and the recombinant chromosomes on the expression of Sb^V. Without exception the recombinant chromosomes that retain the two variegating junctions of the original suppressing w^{mMc} chromosome also retain the full suppressing ability of the parental w^{mMc} chromosome. Exchanging any or virtually all of the euchromatic portions of the two chromosomes (double recombination events) had no effect on Sb^V. The average expression of *Stubble* in the presence of the w^{mMc}-bearing recombinant chromosomes is 95 ± 3% *vs.* 91 ± 2% for the parental w^{mMc} chromosome. Likewise the reciprocal recombinants, those containing various portions of the central euchromatic region of the parental w^{mMc} chromosome but with normal euchromatic-heterochromatic junctions, did not show any suppression of Sb^V. The average expression of *Stubble* induced by the nonvariegating recombinant chromosomes was 69 ± 6% *vs.* 67 ± 2% for the parental *y cv v f* chromosome. Hence, the ability of the w^{mMc} chromosome to suppress Sb^V comaps with heterochromatic/euchromatic junctions and suggest that one or both of these regions, and not a second site suppressor mutation, was responsible for the interaction between w^{mMc} and Sb^V.

The preceding experiments localized the suppressing effect to the abnormal eu-heterochromatic boundaries. It was of interest to determine whether the suppression mapped to the proximal boundary of the variegating chromosome, which is responsible for the *white* variegation, or to the distal boundary, or is partitioned between them. This was determined by generating recombinant chromosomes between the marked w^{mMc} chromosome that suppresses Sb^V and the w^{m4} chromosome, which has no, or only a slight, effect on Sb^V (Figure 2C). Recombinant strains bearing the w^{mMc} proximal junction and the w^{m4} distal junction and their reciprocal partners were tested for their effect on Sb^V. In 65 independent recombinant w^{mMc}-w^{m4} chromosomes bearing either the w^{mMc} proximal and the w^{m4} distal junctions, or the converse (as well as various sections of marked euchromatin) generated from 14 independently derived w^{mMc} recombinant chromosomes, the suppressing effect segregated exclusively with the w^{mMc} proximal junction (data not shown). The average expression of Sb^- phenotype in Sb^V for all recombinants with the w^{mMc} proximal-w^{m4} distal junction was 91 ± 4%; whereas the average for all recombinants with the w^{m4} proximal-w^{mMc} distal chromosomes was 75 ± 6%. The latter value was slightly higher than expected (67 ± 2%) for an unmodified Sb^V, but not statistically significant. It is possible that the w^{m4} proximal junction or that the w^{mMc} distal boundary does have a slight effect on Sb^V. Nevertheless the values from the two recombinant classes are clearly distinct. Thus the w^{mMc} proximal junction is responsible for all, or nearly all, of the suppression of Sb^V and clearly the source of the distal junction in not important in determining the interaction.

DISCUSSION

Simultaneous variegation for euchromatic and heterochromatic genes: In this study we demonstrate interactions between chromosomal rearrangements undergoing PEV. We interpret these interaction phenotypes as competition for limited chromatin proteins. This competition results in a redistribution of chromatin proteins that influences gene packaging and thus gene expression. The redistribution of heterochromatic components, which is suggested by the phenotype of PEV, usually cannot be monitored genetically because it requires variegating reporter loci flanking each

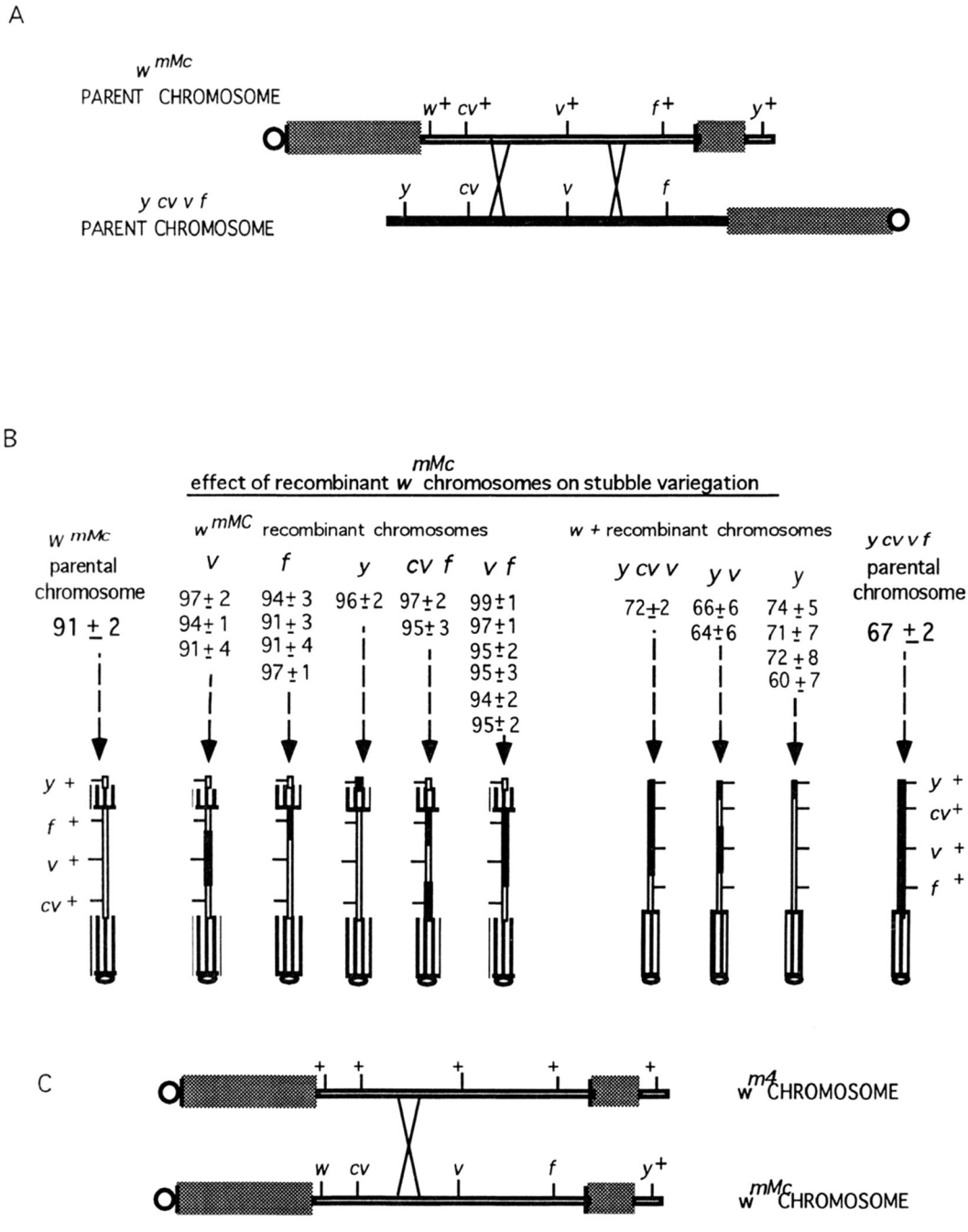


FIGURE 2.—Mapping the suppressing ability of the w^{mMc} chromosome on *Stubble* variegated. (A) Schematic diagram of double crossover used to substitute various euchromatic portions of the original w^{mMc} chromosome. The thin open line represents euchromatin of the w^{mMc} chromosome; the thin solid line represents euchromatin of the *ycvvf* chromosome and the box represents heterochromatin. (B) Diagram of recombinant chromosome. The effect on *Stubble* variegation is shown above. (C) Schematic diagram of recombination events between the marked w^{mMc} chromosome and the w^{m4} chromosome used to generate the w^{mMcL}-w^{m4R} and the w^{m4L}-w^{mMcR} chromosomes. The thin open line represents euchromatin and the boxed lines represents heterochromatin.

breakpoint. However, the lt^{x13} rearrangement is broken sufficiently close to the heterochromatic gene *light* that variegation for this gene can be observed. By combining this rearrangement with a homologue that carries recessive alleles of genes located near the euchromatic breakpoint, we have demonstrated that the expression of both heterochromatic and euchromatic genes are altered concomitantly. This disrupted expression is presumably due to the movement of heterochromatic components from the normally heterochromatic chromosomal segment into formerly euchromatic regions. Thus, the organization and location of the protein components that make up heterochromatin seems to be relatively labile.

This type of coincident disruption is unlikely to be a peculiarity of the lt^{x13} rearrangement. There are a number of rearrangements that have been reported to variegate for both heterochromatic and euchromatic genes. The rearrangements $In(2LR)^{Rev}$ and *Plum* (=$In(2R)bw^{V1}$) variegate for both the heterochromatic gene *light* and the euchromatic gene *Revolute*, and *brown*, *minus*, and *abbreviated*, respectively (DUBININ 1936; SCHULTZ 1941; LINDSLEY and ZIMM 1992). In addition, $T(2;4)ast^{v}$ (BELYAEVA *et al.* 1993), *T(3;4)684* (DUBININ and SIDEROV 1935), and w^{mt11} revertants (PANSHIN 1938) variegate for *cubitus interruptus* and the euchromatic genes *asteroid*, *hairy*, and *white*, respectively, while $In(1)sc^{S1}$ variegates for the ribosomal RNA genes and *scute* (NIX 1973).

Finally, a similar situation has been observed cytologically by PROKOFYEVA-BELGOVSKAYA (1947) who showed that inactivation of the rearranged *white* gene in *In(1)w*^{m4} was coupled to decondensation and adoption of a more euchromatic morphology of the terminally stranded section of heterochromatin. Thus variegation of heterochromatic genes is probably a reflection of generalized changed in the structure of the heterochromatin or in the position of this heterochromatic region within the nucleus.

Interactions between different variegating rearrangements: Only limited information about the distribution of heterochromatic proteins can be gleaned using single-variegating rearrangements. Such experiments do not distinguish between local alterations in chromatin and a more global redistribution among chromosomes. If the presence of one variegating chromosome can influence the level of variegation of another, unlinked variegating rearrangement, this would indicate both competition for and transfer of heterochromatic protein components, based perhaps on a hierarchy of association kinetics and/or DNA replication and chromatin assembly times.

We examined individuals containing two or more variegating rearrangements and compared the expression of the variegating reporter genes in these individuals to their counterparts in single-variegating strains. Conceptually, two variegating rearrangements might either fail to interact, interact reciprocally, or exhibit nonreciprocal interactions. Of the nine pairwise combinations between different variegating rearrangements described in this study, all but two or three exhibited interactions. A plausible hypothesis to account for the influence of one variegator upon another invokes competition between the two rearrangements for components of heterochromatin that are limited in amount. In principal, the nature of the interaction should indicate which portions of the genome are affected. Several previous studies have described interactions between different pairs of variegators. In three cases the variegators behaved independently, analogously to *w*^{m4} and *bw*^{V} in this study (BISHOP 1992; BELYAEVA *et al.* 1993). Two instances of reciprocal interactions have also been reported (SCHULTZ 1941; WARGENT and HARTMANN-GOLDSTEIN 1974). The nine combinations reported in this work in addition to those previously reported define all of these possible modes of interactions and are summarized in Table 5. The excess of cases in which two rearrangements did compete with each other indicates that the genome has considerable latitude in the reorganization of the protein components of chromatin, at least during some cell cycles. These results also suggest considerable sharing of components between the heterochromatin of different variegating rearrangements and thus different genomic regions. A similar conclusion has been inferred from the fact that most dominant mutations that suppress or enhance PEV act similarly on number of different variegating loci (REUTER *et al.* 1982b; SINCLAIR *et al.* 1983, 1989; BISHOP 1992).

Previous studies have focused on suppression of variegation associated with either the presence of an extra *Y* chromosome or mutations that suppress PEV. These conditions alter either the total amount of DNA to be packaged as heterochromatin (extra *Y* chromosome) or decrease the amount of functional chromatin proteins (deletions or mutations in genes encoding protein components of chromatin) and thus alter the stoichiometry of chromatin components. In this study, suppression of PEV at one variegating breakpoint is induced by the presence of another variegating rearrangement. When combining two or more variegating rearrangements there is presumably no net change in either the total amount of DNA in the cell, or the proteins that package it. By default then, the suppressing interactions necessarily result from either a redistribution of chromatin protein components within the genome or by the consignment of one of the rearranged segments to an unpropitious compartment of the nucleus, which concomitantly displaces the other rearrangement to its normal compartment (or an alternate, but more suitable compartment for gene expression).

Similarities between suppression of PEV by the *Y* chromosome and other variegating rearrangements: The addition of an extra *Y* chromosome to the genome will suppress the variegation of any of the rearrangements used in this study. The ability of "extra" heterochromatic material to suppress nearly all variegating rearrangements may be pertinent in formulating a model for the mechanism of competition between variegating rearrangements. ZUCKERKANDEL (1974) proposed that the addition of an extra *Y* chromosome provided a large block of DNA to be packaged as heterochromatin. By virtue of being sequestered on the *Y* chromosome, any protein components that are produced in limited amounts would be unavailable to the variegating chromosome. Therefore, the illicit spread of heterochromatic components at the eu-heterochromatic junction is reduced in distance and/or frequency. While the effect of an additional *Y* chromosome is clearly manifest at the eu-heterochromatic boundary, where the variegating gene is located, it should affect other regions as well. Although it is tempting to postulate that the suppression of a variegating rearrangement by the *Y* chromosome and by another variegating rearrangement occurs by essentially the same mechanism some differences are obvious.

The suppression of PEV caused by the presence of other variegating rearrangements in the genome is both more specific and less extreme than that caused by altering the *Y* chromosome ploidy. The *Y* chromosome affects almost all variegating rearrangements whereas several combinations of variegating rearrangements do not appear to interact. Secondly, addition of an extra

TABLE 5

Summary of interactions between different variegators

Variegator 1	Variegator 2	Phenotype of combination[a] Var. 1	Phenotype of combination[a] Var. 2	Flow of heterochromatic components	Reference
In(1)w^{m4}	*T(2;3)Sb^{V}*	0	0	NO	This paper
In(1)w^{m4}	*In(2LR)bw^{V}*	0	0	NO	This paper
In(1)y^{3P}	*T(2;3)Sb^{V}*	0	0	NO	BISHOP (1992)
Dp(1;f)1337	*T(2;3)ast^{V}*	0	0	NO	BELYAEVA *et al.* (1993)
Dp(1;1)pn2b	*Dp(1;1)pn2b*	0	0	NO	BELYAEVA *et al.* (1993)
In(1)w^{mf}	*T(2;3)Sb^{V}*	0	+/0	From elsewhere to Var. 2	This paper
In(1)w^{mf}	*In(2LR)bw^{V}*	0	+	From elsewhere to Var. 2	This paper
T(2;3)Sb^{V}	*In(2LR)bw^{V}*	0	−	From Var. 2 to elsewhere	This paper
In(1)w^{mMc}	*T(2;3)Sb^{V}*	0	−	From Var. 2 to elsewhere	This paper
In(1)w^{mMc}	*In(2LR)bw^{V}*	0	−	From Var. 2 to elsewhere	This paper
In(1)w^{m51b}	*T(2;3)Sb^{V}*	0	−	From Var. 2 to elsewhere	This paper
In(1)w^{m51b}	*In(2LR)bw^{V}*	0	−	From Var. 2 to elsewhere	This paper
In(1)m	*In(2LR)Rev^{2B}*	+	−	From variegator 2 to variegator 1	WARGENT and HARTMAN-GOLDSTEIN (1974)

[a] +, enhancement of variegation; − suppression of variegation, thus a net addition or loss to that junction; 0, no change in the level of variegation, thus no loss or gain to that junction; Var., variegator; NO, none observable.

Y chromosome suppresses PEV more strongly than does the addition of a second variegating rearrangement. For example, the addition of one *Y* chromosome *vs.* the presence of one *w^{mMc}* chromosome suppresses *Sb^{V}* to 99% *vs.* 91%, respectively, and *bw^{V}* to 103 ± 3% *vs.* 87 ± 6%, respectively. These differences in the ability of the *Y* chromosome and other variegating rearrangements to suppress variegation may have implications for the molecular basis of variegation.

If the suppressive effect of an additional *Y* chromosome is due to binding and cloistering of protein components of heterochromatin, then either one DNA sequence is held in common by the *Y* chromosome and all variegating rearrangements or the *Y* chromosome is a mosaic of heterochromatic "nucleation" sequences, differing numbers of which may be held in common with any given variegating rearrangement. Functional mosaicism of the *Y* chromosome has been inferred from variable suppression of PEV by different regions of the *Y* chromosome (HINTON 1949; BROSSEAU 1964; SPOFFORD 1976). Although DIMITRI and PISANO (1989) have suggested that the *Y* chromosome is functionally uniform, cytogenetic and molecular analysis suggest that at the structural level the *Y* chromosome is nonuniform. Functional nonequivalence of centric heterochromatin has also been inferred from different responses of variegating rearrangements in different suppressor mutation backgrounds (BISHOP 1992). Simple sequence repeats, which are a major DNA sequence component of heterochromatin, show a nonuniform distribution in heterochromatin (BONACCORSI and LOHE 1991; LOHE *et al.* 1993) and recent work by DORER and HENIKOFF (1994) suggests that these multiple repeats might act as nucleation sites for heterochromatin formation. These studies and the results of the interaction between variegating rearrangements all support a mosaic model of heterochromatin function and structure in which the suppression of one variegating rearrangement by another functions much as suppression by the *Y* chromosome but with a more limited set of DNA sequences to act as binding or nucleation sites for heterochromatic proteins.

Involvement of nonvariegation regions of the genome: The nature of the interactions between different variegating rearrangements provides insight about which parts of the genome are involved in transfer of heterochromatic components. For example, in the case of reciprocal effects (*i.e.*, the gene inactivation of one variegator is suppressed while the other is enhanced) heterochromatic components may be either transferred directly from one variegating junction to the other or compete from a common pool. In the cases of suppression or enhancement of one rearrangement in the absence of a response by the other, other parts of the genome are necessarily involved as a repository for heterochromatic components. These sites from which heterochromatic protein components could be drawn might be either undisturbed (nonrearranged) regions of the genome or the smaller, fragmented segment of heterochromatin which is repositioned into a euchromatic region of the genome. One might expect this fragment of heterochromatin to be destabilized as a consequence of being relocated into euchromatin. However, this did not seem to be the case with the *white* variegating chromosomes used in our experiments (*w^{m4}*, *w^{mf}*, *w^{mMc}*, and *w^{m51b}*). For example, the exchange

of the "marooned" distal segment of w^{mMc} heterochromatin with the homologous segment from the nonsuppressing w^{m4} rearrangement did not affect the ability of w^{mMc} to suppress Sb^V. Thus it appears that other parts of the genome are necessarily involved in redistribution or repatterning of chromatin at the altered euchromatic-heterochromatic interface. Similar conclusions have been derived from the generalized lethal effects of extra *Y* heterochromatin (COOPER 1956) and sodium butyrate (REUTER *et al.* 1982a).

Implications of the competition results for models of PEV: The observation that one variegating rearrangement can suppress another in an otherwise euploid cell has implications for the nuclear compartmentalization model of PEV. This model argues that variegation results from aberrant localization of genes within the nucleus (TALBERT *et al.* 1994). According to this hypothesis, a rearranged gene will variegate because the rearrangement brings it into a region of the nucleus that is relatively devoid of transcription factors necessary for the expression of the reporter gene. The suppressing activity of extra heterochromatin has been explained as a steric effect that occludes the heterochromatic compartment so that the variegating chromosome is relegated to a compartment more suitable to its activity (EBERL *et al.* 1993). Since there is no change in the total amount of DNA or (chromatin-packaging protein) in the cell, the various patterns of interaction between variegating rearrangements is somewhat difficult to reconcile with this model. The bending of one chromosome rearrangement might sterically interfere with bending and repositioning of another rearrangement. This might account for the nonreciprocal interactions that we see between two variegators. For example, if the gene inactivation seen in PEV results from the consignment of one variegator to an ill-suited compartment, then one might argue that the repositioning of one variegator to this compartment effectively precludes the second variegator from occupying same compartment. As a consequence, perhaps the second variegator would now occupy a compartment more favorable to the expression of its variegation reporter gene or genes (*i.e.*, suppression of variegation). The observations that two variegating rearrangements act independently when combined in the same cell might be accounted for by hypothesizing that the two variegating rearrangements cohabit in a particular compartment. But it is difficult to reconcile this argument with the nonreciprocal interactions in which one posits that one variegator effectively displaces a second variegator from occupying the same compartment. Alternatively, one might argue that two variegating rearrangements, which act independently, occupy different compartments, but these nuclear compartments should be present in rather limited numbers. It is more difficult to explain how reciprocal interactions occur (enhancement of one variegating rearrangement coupled with suppression of another). Finally, since there should be a rather limited number of these nuclear compartments, one might suppose that the presence of three or four variegating rearrangements in a single strain might dramatically alter consignments to specific compartments, and this was not observed. While we favor the model of PEV in which there is a competition for a limited number of chromatin proteins, our data certainly do not exclude the model in which PEV occurs as a consequence of repositioning the variegating segment of the genome into a nuclear compartment that does not allow efficient transcription of the variegating genes. Indeed, these two models need not be mutually exclusive. One might argue that relocating the variegating segment of the genome to a new nuclear compartment might alter both the timing of the replication and the packaging of its DNA. In addition, the mosaic phenotype of PEV might result from more than one molecular mechanism.

A more trivial explanation for the results we obtained might be that suppression of one variegating rearrangement by another is due to variegation of a locus located near the rearrangement breakpoint, which itself acts as a suppressor or enhancer of PEV. Loci that modify PEV have been found near the breakpoint of the Sb^V rearrangement (SINCLAIR *et al.* 1983; REUTER *et al.* 1986). However, these modifiers would necessarily have act only on a small, and very specific, subset of other rearrangements. This would appear to require a rather special contrivance, especially in light of the observation that of more than 25 different *Su(var)* loci studied to date, all act on a wide spectrum, if not all, variegating rearrangements examined. Furthermore, the most potent suppression effect observed in these competition experiments, the w^{mMc} rearrangement, maps to the proximal *X* chromosome, a region not known to encode loci which modify PEV. Although the existence of variegating loci, which themselves act as suppressors or enhancers of specific variegating rearrangements, is difficult to exclude, this is not the most parsimonious explanation of the ability of one variegation rearrangement to suppress another.

The four *white* variegating strains (w^{m4}, w^{mJ}, w^{mMc}, and w^{m51b}) used in our studies share similar euchromatic breakpoints, yet they differed in their ability to compete with both Sb^V and bw^V. This suggests that the heterochromatic, rather than the euchromatic portion of the genome is the important determinant of the degree of competition. Interestingly, among these four *white* mottled variegators, the ability to suppress variegation of Sb^V, in w^m-Sb^V combinations, seems to correlate with the distance over which heterochromatin spreads in these w^m rearrangements (V. LLOYD, unpublished observations). The distance of spread is likely to be a property of the type of heterochromatic sequence at, or near, the variegating junction. The nature and properties of these DNA sequences that determine the "attrac-

tiveness" for certain protein components of heterochromatin or that determine its positioning within the nucleus is likely to be complex. The difference in the ability of different variegating rearrangements to compete might suggest that heterochromatin differs only in the number and/or types of binding sites for heterochromatin proteins (or nuclear matrix, if position is more important). However, recent results show that variegation of the *light* and *white* genes in the lt^{x13} rearrangement is induced by different heterochromatic sequences (HOWE *et al.* 1995). This suggests that there is considerable diversity in the sequence of DNA elements that induce variegation. Qualitatively or quantitatively different types of heterochromatin may exist within a cell, determined perhaps by preferential utilization of some components of heterochromatin by virtue of replication timing and/or kinetics of chromatin assembly. In the face of such complexity, molecular analysis of these sequences remains difficult. Identifying sets of rearrangements that compete with each other, should define chromosomal regions that function similarly. This should be useful in assessing the functions of molecularly defined heterochromatic sequences.

In summary, we have shown that different variegating rearrangements can interact when combined in one cell. We interpret this interaction as a redistribution of the protein components of heterochromatin, since there is no change in the total amount of DNA or dosage of the loci that encode proteins that package chromatin. The fact that the redistribution is not restricted to the one variegating chromosome, and that the competition can be nonreciprocal suggests that other, nonvariegating, portions of the genome are affected by the process of variegation. The results of this study also suggest that heterochromatin is functionally mosaic. Finally, these results are most easily accommodated by a model of PEV in which the variegation results from redistribution of chromatin components, leading to altered compaction of various DNA sequences.

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