NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

THE UNIVERSITY OF ALBERTA

ISOLATION AND CHARACTERIZATION OF MUTATIONS THAT MODIFY POSITION EFFECT VARIEGATION IN *DROSOPHILA MELANOGASTER*

BY

DEQUAN KONG

A thesis submitted to the Faculty of Graduate studies and Research in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE.

DEPARTMENT OF GENETICS

Edmonton, Alberta

Fall, 1991

ISBN 0-315-69955-8

THE UNIVERSITY OF ALBERTA

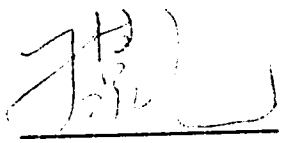
RELEASE FORM

NAME OF AUTHOR: DEQUAN KONG

TITLE OF THESIS: ISOLATION AND CHARACTERIZATION OF MUTATIONS THAT MODIFY POSITION EFFECT VARIEGATION IN *DROSOPHILA MELANOGASTER*

DEGREE: MASTER OF SCIENCE

YEAR THIS DEGREE GRANTED: 1991



Dequan Kong
11025-Sask. Drive
Edmonton, Alberta
Canada T6G 2B4

Date October 10, 1991

"This is only the first step in a ten-thousand-mile long march"

---- Chairman Mao

DEDICATED

TO MY PARENTS AND MY DEAR YANG GUANGZHEN,
FOR THEIR LOVE AND ENCOURAGEMENT

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and research for acceptance, a thesis entitled ISOLATION AND CHARACTERIZATION OF MUTATIONS THAT MODIFY POSITION EFFECT VARIEGATION IN *DROSOPHILA MELANOGASTER* submitted BY DEQUAN KONG in partial fulfillment of the requirements for the degree of Master of Science.

(Supervisor) Dr. John Locke

(Committee Chairman) Dr. Allen Good

(Committee Member) Dr. Michael Russell

(Committee Member) Dr. John Bell

(Committee Member) Dr. Roseline Godbout

Date

The phenomenon of position effect variegation (PEV) in Drosophila provides a model system to study heterochromatin in eukaryotes. PEV is seen in mutations that result in the mosaic expression of an affected gene. The mosaic or variegated expression is due to a chromosomal rearrangement, which brings a euchromatic gene adjacent to heterochromatin. In this location, the euchromatic gene expression is influenced by the heterochromatin which inactivates this euchromatic locus in some somatic cells but not in other cells, thus giving a mosaic expression. The extent of variegation in these mutations can be altered by the second site dominant suppressor or enhancer mutations found elsewhere in the genome. These modifier genes are thought to encode proteins which inhibit or promote the formation of heterochromatin at the variegation breakpoint. Therefore, studies on these genes may throw some light on the structure and function of heterochromatin.

By using the pUChsneo P-element in combination with a stable transposase-producing Δ2-3 P-element as a "mutagen", 66 dominant suppressor mutations of position effect variegation, representing at least 30 independently isolated mutations, were induced. In addition, 4 enhancer mutations were derived from unstable suppressor mutation stocks. Of these mutations, 20 suppressor and 4 enhancer mutations were given a detailed characterization.

All of these mutations can modify two different variegating alleles, w^{m4} and B^{sv}, indicating that they probably have a general effect on position effect variegation. Most of the suppressor and all

four enhancer mutation-bearing chromosomes are lethal as homozygotes. Some of them are recessive semi-lethal and the homozygous viable mutations are generally recessive sterile for males and (or) females. But at this time, it is still not clear whether these effects are associated with the mutations or not. Genetical studies showed that 6 of 20 suppressor and all four enhancer mutations were located on the second chromosome while 14 were located on the third chromosome. Complementation analyses of 9 third chromosome recessive lethal mutations identified four lethal complementation groups. In addition, one enhancer and three suppressor mutations were genetic mapped. Cytogenetic studies of polytene chromosomes from 1 enhancer and 20 suppressor mutations revealed no visible chromosomal aberration except that for Su(var)KD341 about 95% of the 3L and 3R tips were attached to each other forming a "ring". Careful examinations of 3L and 3R tips revealed no visible change in their banding patterns, indicating that the attachment of 3L and 3R may be due to a high frequency of "ectopic pairing".

Southern analyses of Su(var) mutant DNAs revealed that only Su(var)KD340 contained an intact pUChsneo P-element. In addition, I found, for the first time, that the parental source of w^{m4} can affect the expression of w^{m4} variegation in the progeny.

Acknowledgments

I would like to express my deep appreciation to my supervisor, Dr. John Locke, for his patient instruction, understanding and all the help that he gave to me throughout the period of this study.

I thank my supervisory committee members, Dr. M. Russell and Dr. J. Bell, for their valuable comments and suggestions.

I am very grateful to Scott Hanna for his time and effort in rearing the stocks needed for this research and teaching me how to recognize the phenotypes of various fly mutations.

Special thanks to my friend Wang Tongyan, who is in the Department of Statistics, for helping me with the statistical analysis with the University of Alberta computer system.

Table of Contents

List of Tables

List of Figures

ABBREVIATIONS

Amp^r	Ampicillin resistance
bp	base pair
Ci	Curie
D. melanogaster	*Drosophila melanogaster*
D. virilis	*Drosophila virilis*
dATP	2'-deoxyadenosine 5'-triphosphate
dCTP	2'-deoxycytidine 5'-triphosphate
DNA	Deoxyribonucleic acid
dTTP	2'-deoxythymidine 5'-triphosphate
EDTA	Ethylene Diamine Tetra Acetic acid
EMS	Ethyl Methane Sulphonate
EtBr	Ethidium Bromide
dGTP	2'-deoxyguanosine 5'-triphosphate
HCl	Hydrochloric acid
kb	kilobase
KCl	Potassium Chloride
LB	Luria-Bertani medium
$MgCl_2$	Magnesium Chloride
$MgSO_4$	Magnesium Sulphate
mmol	millimole
NaCl	Sodium Chloride
NaH_2PO_4	Sodium phosphate monobasic
NaOH	Sodium Hyhroxide
NHCp	Nonhistone Chromosomal Proteins

PEV	Position Effect Variegation
RNA	Ribonucleic Acid
Rsp	Responder
Rsp^r	Responder resistant
Rsp^s	Responder sensitive
SCS	Specialized Chromatin Structure
SD	Segregation Distorter
SDS	Sodium Dodecyl Sulphate
SSC	Sodium Chloride, Sodium Citrate
TE	Tris-EDTA buffer
Tris	THAM-Tris (Hydroxyethyl) amino methane
µg	microgram
µl	microliter
π	Pi, a wild type *D. melanogaster* lab stock

I. Introduction

The genetic material of eukaryotic cells is packaged into chromatin, which contains DNA, histones and nonhistone chromosomal (NHC) proteins. The structure of chromatin has been implicated in many genetic functions, such as transcription, replication, recombination and segregation of the genes (for reviews see, Elgin, 1975; McGhee and Felsenfeld, 1980; Van Holde, 1989). The complexity of chromatin makes it difficult to study both its function and structure. However, position effect variegation (PEV) in *Drosophila melanogaster* provides a good system for studying these processes and identifying structural components of chromatin.

Variegating position effects are a class of mutants that result from chromosomal rearrangements, which bring a euchromatic gene adjacent to heterochromatin. A variegated phenotype is produced because heterochromatin is thought to inactivate adjacent euchromatic genes in some somatic cells but not in other cells giving a somatic mosaic. The phenotype of variegating mutations can be altered by the presence of dominant suppressor and enhancer mutations located elsewhere in the genome. These genes have been thought to encode some proteins which affect the formation of heterochromation (reviewed by Eissenberg, 1989; Henikoff, 1990). Therefore studies on these genes may throw some light on the structure and function of heterochromatin.

Although many modifying loci have been identified and mapped both genetically and cytogenetically (for review see

Henikoff, 1990), few of the genes have been cloned and studied at the molecular level (Reuter *et al.*, 1990; Eissenberg *et al.*, 1990). Most of these genes have not been cloned and their gene structure, organization and function remain unknown. In order to clone some modifying genes, and therefore study their structure and function at the molecular level, pUChsneo in combination with transposase-producing P-elements mutagenesis was used to induce mutations. This research reports the isolation and characterization of more than 20 independently-isolated suppressor mutations and four enhancer mutations.

1. Organization of Chromatin

One of the main differences between eukaryotic and prokaryotic cells is that the genetic material (DNA) in eukaryotic cells is organized into chromatin, which is a complex between DNA, histones and non-histone chromosomal (NHC) proteins. Changes in chromatin structure are thought to play a fundamental role in many genetic processes, especially gene activation and repression (for reviews see, McGhee and Felsenfeld, 1980; Elgin, 1975; Van Holde, 1989).

Each individual eukaryotic chromosome contains a single DNA molecule. This single long DNA molecule must be packaged, in an orderly way, in the relatively small cell nucleus. Since the 1970s, it has been well accepted that the nucleosome is the primary packaging unit of chromatin (McGhee and Felsenfeld, 1980). The amount of DNA associated with each of the nucleosome beads is approximately

200 base pairs. Of these 200 base pairs of DNA, 146 base pairs are wrapped around the nucleosome core particle, which contains two each of histones H2A, H2B, H3 and H4. The single molecule of histone H1 is associated with about 60 bp of linker DNA. When chromatin was treated to unfold its higher-order packing, it appeared as a "beads-on-a-string" structure under the electron microscope.

In living cells, the beads-on-a-string structure is only the first order of packaging form of DNA into chromatin. When interphase nuclei are gently lysed, most chromatin is seen as a 30 nm filament in which the nucleosomes are further assembled into a higher order structure (Finch and Klug, 1976). Different models have been proposed for the packing of nucleosomes in the 30 nm chromatin fiber. However, most evidence strongly favors the solenoid model, in which the beads-on-a-string are thought to be coiled to form a solenoid-like structure. The solenoid contains six nucleosomes per turn. Histone H1 molecules seem to be responsible for packing the nucleosome into a 30 nm fiber (for review see Felsenfeld and McGhee, 1986).

Possibly the next level of folding of the DNA into chromatin is the formation of looped domains (Stubblefield and Wray, 1971; Paulson and Laemmli, 1977). It has been assumed that the looped domains in chromatin are established and maintained by DNA-binding proteins that clamp two regions of the 30 nm fiber together, presumably by recognizing specific DNA sequences that will form the neck of each loop. Moreover, it has been suggested that each domain defines a unit of genetic function (Vdvardy et al, 1985). When these

genetic units are inactive, the chromatin fiber of the domain would be highly condensed. On the other hand, when a gene or genes within a domain become active, the chromatin fiber of the domain would become decondensed to give a more relaxed or looped out structure (for review see Eissenberg *et al.*, 1985). In support of these views, Vdvardy, *et al.* (1985) found two possible domain boundary sequences (SCS and SCS' specialized chromatin structures) either of the side of 87A7 heat shock locus in *Drosophila melanogaster*. Kellum and Schedl (1991) found that these sequences can insulate a reporter *white*$^+$ gene against chromosomal position effects. In order to fit into the cell nucleus, the DNA probably needs to be coiled at another level.

2. Heterochromatin

a). Origin and Classification

Heterochromatin was first defined by Heitz (1928). He coined this term to describe chromosome segments, that maintain a highly compacted state during the interphase of the mitotic cell cycle, and therefore remain visible in the light microscope when stained with some dye during interphase. When carefully following chromosomes through successive cell division cycles, Heitz (1928) found that the heterochromatin is actually due to the non-decondensation of chromatin at telophase of the mitotic division. In contrast, euchromatin condenses during mitosis but is decondensed after telophase. The heterochromatin that Heitz (1928) defined was later

called constitutive heterochromatin, since in the 1960's another form of heterochromatin (facultative) was defined by Brown (1966).

As we know, in mammals with X/Y systems of sex determination, the X-linked genes must be dosage compensated. In the 1960's it was found (Stern, 1960) that in female mammalian cells, one of the X chromosomes becomes condensed (Barr body) and inactivated during early embryonic stage and then remains inactive over many cell generations in all or many somatic cells. Subsequently, Bernard (1988) argued that the original definition of heterochromatin proposed by Heitz (1928) was not quite correct. Bernard (1988) classified the condensed chromatin within interphase nuclei into three categories.

1) Tissue-specific condensed euchromatin. He argued that this condensed chromatin is actually euchromatin in some specific cells. The condensation and decondensation of this particular portion of euchromatin in differentiated cells is a kind of tissue-specific transcriptional control system. This type of inactivation is often reversible.
2) The facultative heterochromatization of euchromatin. This form of chromatin corresponds to facultative heterochromatin.
3) Constitutive heterochromatic regions. These are the regions to which Heitz (1928) originally gave the name heterochromatin.

After more than 60 years of study, heterochromatin still remains a major biological enigma (for reviews see Hannah, 1951; Bernard, 1988). Since many cases of Position Effect Variegation (PEV) are associated with constitutive heterochromatin, in the following review I am going to concentrate on some important properties of constitutive heterochromatin.

b). DNA Component of Heterochromatin

Heitz's original definition of heterochromatin was based on the observations of interphase nuclei. At metaphase, euchromatin and heterochromatin are equally condensed, while at interphase, the heterochromatin is more condensed and remains visible under the light microscope. Since the development of various chromosomal banding techniques, it has been found that the constitutive heterochromatic regions usually give a positive C-banding staining pattern. Interphase chromocenters formed from constitutive heterochromatic regions also can be stained by C-banding (Arrighi and Hsu, 1971).

The use of *in situ* hybridization techniques showed that the heterochromatic regions are composed mainly of highly repetitive (hr) DNA sequence (Sueoka, 1961). However, constitutive heterochromatin is not composed entirely of hr DNA. hr DNA is also located in the regions other than heterochromatin. It seems that there is a good correspondence between the location of hr DNA and the C-banding staining sites.

By using tritium-labeled thymidine ([^{3}U] TdR) as a probe, Lima de Faria *et al.* (1968) found that heterochromatin regions were late replicating, although not all late replicating regions were necessarily heterochromatin. In the polytene chromosomes of *Drosophila melanogaster*, the heterochromatin remains underreplicated and forms a chromocenter.

c). Heterochromatin in *Drosophila melanogaster*

In *Drosophila melanogaster* the entire Y chromosome and the proximal half of the X chromosome is heterochromatic (Heitz, 1933; Hannah, 1951). Approximately a quarter of each of the arms of chromosomes 2 and 3 are also heterochromatic. However, the length of the major heterochromatic regions in a salivary gland chromosome of *Drosophila melanogaster* become relatively much shorter than that in the somatic chromosomes, due to the underreplication of heterochromatin. The heterochromatin of salivary gland chromosomes in *Drosophila melanogaster* has been classified into four categories (Hannah, 1951). 1) The heterochromatin of the Y chromosome and the large block near the centromere of the X and the autosomes, which fuse together into a common mass in the chromocenter and retain no definite structure. This class corresponds to the α-heterochromatin termed by Heitz (1934). 2) The heterochromatin which retains some structure and forms poorly banded segments. 3) The nucleolus-associated heterochromatin. The class 2 and class 3 correspond to the β-heterochromatin of Heitz (1934). 4) The possibly existing intercalary heterochromatin.

d). Genes Located on the Heterochromatin

Very soon after Heitz (1928) defined heterochromatin, it was found that the heterochromatic regions in Drosophila chromosomes could be roughly correlated with the so-called genetically inert regions (Muller and Painter, 1932) since very few genes had been found in these regions. The later discovery that heterochromatin is

mainly made up of highly repetitive DNA sequences seems consistent with this concept (Peacock *et al.*, 1977). However, extensive genetic studies of Drosophila heterochromatin have shown that it contains several different classes of genes (Hilliker and Appels, 1980). One class includes genes which are members of a repetitive gene family, such as 18S and 28S ribosomal genes, ABO (Pimpinelli *et al.*, 1985) and Responder locus (Pimpinelli and Dimitri, 1989). Another class of genes has been identified within or very close to the centromeric heterochromatin of chromosome 2 and 3. At least 21 genes of this class have been identified. It has been suggested that this class of genes is functionally unique (contain unique sequences) and may be structurally similar to conventional euchromatic genes, since they are mutable by ethylmethane sulfonate (EMS) and P-elements (Hilliker, 1976; Devlin *et al.*, 1990).

Not only are many genes located in the heterochromatic regions, but also the correct expression of these genes is dependent on their location in or near heterochromatin. The *light (lt)* gene is located in the centromeric heterochromatin of chromosome 2 (40B-F of the polytene chromosome map) of *Drosophila melanogaster.* Chromosome 4 of *Drosophila melanogaster* has the gene *cubitus interruptus* located in its heterochromatin. *Peach* is a gene in *D. virilis* located near the centromeric heterochromatin of chromosome 5. When these genes were brought into euchromatic regions by chromosomal rearrangements, variegated expression of these genes is observed (Dubinin and Sidorov, 1934; Schultz, 1936; Lewis, 1950; Baker, 1953; Hessler, 1958). Opposite to the euchromatic gene

position effect variegation, the variegation of heterochromatic genes can be enhanced by an extra Y chromosome and suppressed by loss of a Y chromosome.

e). Possible Functions for Heterochromatin

Since heterochromatin is present in most eukaryotic organisms, it has been assumed that there must be some important cellular functions related to heterochromatic regions. As we know, in most cases the heterochromatin is found near the centromere. One of the earliest suggestions was that such heterochromatin was associated in some way with centromere activity. The validity of this speculation is still not clear, since various results of different people are contradictory (for review see Bernard, 1988).

Several people, including Hilliker and Appels (1980) and Manuelidis (1982) have speculated that heterochromatin may play an important role in determining the three-dimensional structure of the interphase nucleus and so may affect gene expression both directly and indirectly. An ordered arrangement of chromosomes in interphase nuclei has also been proposed as a means by which genetic activity is coordinated within a genome. The existence of compound chromocenters *via* ectopic pairing has contributed to the opinion that an ordered chromosome arrangement is partially attributable to nonspecific association of heterochromatin.

Recently, a heterochromatic satellite DNA was found to be associated with a well-known phenomenon, Segregation Distortor (SD) in *Drosophila melanogaster* (Wu *et al.*, 1988; 1989). The

Segregation Distortor (SD) system represents a well-known example of meiotic drive (for review see Sandler and Golic, 1985), which is the excess transfer to the sperm in a heterozygous male of one of the two homologous chromosomes. There appear to be two main loci (Sd and Rsp) involved in the Segregation Distortor phenotype (Charlesworth, 1988). Segregation distortor (*Sd*), located at 37D2-6 in the euchromatic region of 2L, acts in *trans* to promote segregation distortion. Responder (Rsp), located in the proximal centromeric heterochromatin of 2R, is a *cis*-acting element, which has different alleles showing varying sensitivity to the effects of Sd (Ganetzky, 1977). It has been suggested that segregation distortion occurs when the product of the distortor gene Sd interacts with sensitive alleles (Rsp^s) at the Responder locus carried by a non-SD second chromosomes, causing Rsp^s sperm to develop abnormally (Charlesworth, 1988).

The Responder (Rsp) locus has been cloned and sequenced. It was found that an A-T rich, 120-base pair repetitive sequence was always associated with the Responder locus. Rsp^r (Rsp resistant) strains have only 25 copies on average, whereas a typical Rsp^s (Rsp sensitive) chromosome carried 700 copies. It has been suggested by Wu *et al.* (1988) and Charlesworth *et al.* (1988) that the repetitive sequence is the target for the product of Sd and the interaction of Rsp with the Sd product causes aberrant chromatin condensation in spermiogenesis, resulting in dysfunction of sperm carrying Rsp. Since the presence of these highly repetitive sequences in the Rsp locus causes the low frequency transmission of the chromosome

bearing it when the homologous second chromosome is a Segregation Distortor (Sd) chromosome, why has this highly repetitive satellite DNA sequence not been eliminated? In order to answer this question, Wu *et al.* (1989) compared the fitness of individuals with this family of satellite DNA to those without it. They found that the deletion of Rsp satellite DNA caused a fitness reduction of flies. From this result, they argued that other satellite DNAs probably have a similar positive function but not the dramatic negative effects associated with Rsp.

As we know, the heterochromatinization of one of X chromosomes in female mammals provides a mechanism for dosage compensation. Is the formation of constitutive heterochromatin a way of regulating the gene expression in eukaryotic cells? The complexity of chromatin and heterochromatin structure makes it very difficult to answer this question directly. However, since position effect variegation involves the inactivation of euchromatic genes by heterochromatin it will provide a good system for studying these processes.

3. Position Effect Variegation (PEV)

The position of a gene on the chromosome may affect its expression; this is known as a position effect, and it has been well studied in *Drosophila melanogaster* and has been demonstrated in other organisms (for reviews see Dobzhansky, 1936; Lewis, 1950; Baker, 1968; Spofford, 1976; Eissenberg, 1989; Henikoff, 1990). Lewis (1950) classified position effects into two categories. One was

referred to as stable or S-type position effects and the other was called variegating or V-type position effects.

Stable position effects (S-type) constitute a group of position-effects, in which the change in gene action is somatically stable and is usually not associated with chromosomal aberrations involving heterochromatin. The classical example of S-type position effects was given by Sturtevant (1925). He found that two additional *Bar* regions (16A) located on the same chromosome produce a stronger effect on the eye size than the same two additional regions located on separate chromosomes.

Variegating position effects (V-type) result in the expression of an affected gene as a somatic mosaic. The variegated expression is usually due to a chromosomal aberration (inversion or translocation), which brings the affected euchromatic gene adjacent to heterochromatin. This repositioning permits the heterochromatin to inactivate adjacent euchromatic loci in some somatic cells, but not in other cells, and therefore gives rise to fluctuations in expression (a somatic mosaic).

a). Discovery and Proofs for Position Effect Variegation

Position effect variegation was first described by Muller (1930) under the title of "eversporting displacement". By X-ray mutagenesis, he induced a group of peculiar variants associated with chromosomal aberrations. These variants produced somatic variegation, such as mottled eyes. He also suggested that this phenomenon was caused by either chromosomal or gene instability,

or by an effect on gene action due to an abnormal chromosomal position, perhaps through an interaction with a local gene. In the review of position-effects by Dobzhansky (1936), these "eversporting displacements" were ascribed to a position-effect like Bar. The associated chromosomal rearrangement invariably involved the euchromatic and heterochromatic region of the chromosome (Schultz, 1936).

By obtaining recombinants between the affected locus and the rearrangment breakpoint, which placed the affected gene away from the heterochromatin, Panshin (1935), Dubinin and Sidorov (1935) and Judd (1955) obtained direct evidence that position-effect variegation is not due to the mutation of the affected gene but instead is caused by the chromosomal rearrangements that bring a euchromatic gene next to heterochromatin.

In several other studies, another line of evidence has been obtained. When further chromosomal rearrangements were induced by irradiation, which separated the affected locus from the breakpoint, a new level of variegation can be observed, depending on the new location of the affected genes. Additionally, if the affected gene is moved to a euchromatic region, normal wild type gene action can be restored. This kind of study has been done using *In(1)rst*^{3} (Novitski, 1961) and *bw*^{D} (Hinton and Goodsmith, 1950).

All of these lines of evidence tell us that it is the chromosomal rearrangement itself, that causes the variegation, and not a mutation in the affected gene.

b). Loci Subject to Position Effect Variegation

Most, if not all, loci can exhibit a somatically mosaic expression when brought next to heterochromatin (Spofford, 1976). However, variegated expression can be most easily demonstrated for cell-autonomous loci where a large number of similar cells have the same phenotype (such as the hypoderm or the ommatidia). Of these, the most extensively studied example is the *white* gene (w^+). Each Drosophila compound eye consists of about 800 ommatidia. Each of the ommatidium contains about 12 pigment cells, so very fine mosaicism of eye color can be easily detected. For the white locus, over 35 instances of white-mottled types have been analyzed by studying the rearrangements present in the salivary chromosomes. Without exception, each of these cases was associated with a rearrangement bringing the white locus into close proximity of heterochromatic regions (Schultz, 1936). There is a large amount of variegation variability, which depends on the particular rearrangement being studied, and on the genetic and environmental background. In the case of the white gene in Drosophila, the variegated eye may have a red background color with scattered lighter sectors, a white background with scattered red groups of ommatidia, or it may be of an intermediate color such as cream or pink with darker or lighter patches present. For example, the variegating allele $In(1)w^{m4}$, w^{m4} (hereafter w^{m4}) can often give rise to a "sectored" phenotype (large patches of either all mutant or all wild type cells), sometimes the eye pigment is even distributed depending on the genetic background. Also the pigment of w^{m4} flies

can vary from total mutant white to wild type red. Other mottled alleles of white such as $In(1)w^{m51b}$, w^{m51b} and $In(1)w^{mMc}$, w^{mMc} produce a typical "peppered" pattern, in which darker brown ommatidia (w^-) are scattered throughout the red surface (w^+) of the eye. The mosaic phenotypes related to R(w^+) types have been described in some detailed by Muller (1930), Gowen and Gay (1934), and Demerec and Slizynska (1937).

c). Factors that Modify Position Effect Variegation

The intensity of position effect variegation can be modified by several factors (see review Spofford, 1976).

In general, high rearing temperatures suppress variegation while low temperatures enhance variegation (Kaufmann, 1942; Wargent, 1971; Schalet, 1969). This has been explained by Spofford (1976) by proposing that the temperature effect is due to a shorter time of inactivation of the affected gene at high temperatures. However, Eissenberg (1989) argued that the temperature effect occurs because heterochromatin is somewhat thermolabile, resulting in a later reversal of the inactivation at higher temperatures in some cells.

It was found that additional Y chromosomes suppress variegation while fewer than the normal number enhance it (Gowen and Gay, 1934). By extensive analyses of the suppression effects of different Y chromosomal defficiencies and fragments on three different position effected alleles (V-suppressed lethality, white mottled and brown dominant), Dimitri and Pisano (1989) concluded

that the suppression effect of a Y chromosome is determined by the total amount of Y heterochromatin present in the genome and not by any discrete Y region. Consistent with this result, Eissenberg (1989) suggested that the effect of a Y chromosome on variegation is due to the titration of heterochromatin, since the Y chromosome is heterochromatic in somatic tissue. The heterochromatic Y-chromosome can compete with all other heterochromatic DNA for any dosage-limited proteins or factors responsible for the heterochromatinization of the variegating gene, therefore acting to reduce the probability that euchromatic gene(s) become heterochromatic and inactive (Eissenberg, 1989)

The effects of temperature and additional Y-chromosomes on position effect variegation have been generally regarded as a diagnosis for identifying new mutations showing position effect variegation (Spofford, 1976).

d). Spreading Effects and Clonal Expression of Position Effect Variegation

In order to understand the mechanism of position effect variegation, the following two curious properties of position effect variegation must be considered.

1). Spreading Effects

One of the most peculiar properties of position-effect variegation is the spreading effect. The inactivation of genes can spread from the heterochromatic breakpoint to the nearest gene and then extend to other nearby genes, sometimes as far as about 50 salivary chromosomal bands (Demerec, 1941). This has been

extensively studied by Demerec and Slizynska (1937) and by Schultz (1941) at the white locus when associated with *roughest* and *split* variegation. The genes *rst*+ and *spl*+, which generate a "rough" rearrangement of the eye facets, were located closer to the breakpoint than *w*+ in $T(1;4)w^{m258-18}$. In a variegated eye, the white patches were always rough, but a normally pigmented region could be either rough or not. Therefore, the inactivation of gene spread from *rst*+ to *w*+. When Hartmann-Goldstein (1967) did a very extensive cytogenetic study of a different translocation, $T(1;4)w^{m258-21}$, with the X chromosome break at 3E5-6, it was found that the heterochromatinization of polytene chromosome in the salivary gland cells of flies extended from proximal to distal bands with respect to the rearrangement breakpoint. Band "3C1" was clearly polytenized in more cells than was "3C7", and was always visible when "3C7" was visible. This cytogenetic "variegation" correlated well with the phenotypic variegation of the genes in the *white-Notch* interval.

2). Clonal Expression

Another curious feature of position effect variegation is its clonal nature. As we know, variegation arises when the expression of an affected gene is repressed in some somatic cells but active in others. In eye-pigment variegation in *Drosophila melanogaster*, there are often large areas of the eye that may be either mutant or wild-type, giving rise to patches. By comparing clonal patterns generated by somatic recombination with the eye-pigment patterns of position-effect variegation, Becker (1961; 1966) concluded that

position-effect variegation in mottled eyes has a cell lineage property. The pattern of variegation is determined in the eye anlagen at, or near, the end of the first larval instar when about 16 to 20 presumptive eye cells are present. At this stage, the gene (or genes) near the heterochromatin become inactivated (probably through heterochromatinization) in some cells, but remain activated in other cells. Moreover, the inactivated or activated states of the affected gene (or genes) can been inherited by their progeny cells, and therefore propagated to give rise to patches in the adult eye.

e). Mechanisms of Position Effect Variegation

Since position effect variegation was first described in 1930 by Muller, more than 60 years have passed. In spite of a considerable body of data accumulated on the subject, little is known about the mechanism that leads to mosaicism. Position effect variegation still remains an enigma.

In 1936 when Schultz examined the polytene chromosomes of thirteen different variegating mutants, he found that in every case the euchromatic gene showing a variegating phenotypy was brought next to a block of constitutive heterochromatin. He suggested that euchromatic genes translocated next to heterochromatin were unstable and got lost in some cells. His hypothsis obtained some support after the discovery of the underreplication of heterochromatin in polytene chromosomes. Ananiev and Gvozdev (1974) found that an X chromosome fragment translocated to heterochromatin showed 20% less ^{3}H-thymidine incorporation than

that of the control region, when salivary gland chromosomes were incubated in a solution containing ^{3}H-thymidine for a long time. The same conclusions were obtained after microdensitometry (Wargent *et al.*, 1974; Cowell and Hartmann-Goldstein, 1980). However, definite conclusions can not be made from these experiments since there are two problems with these experiments. First of all, in these experiments cytological and genetical aspects of variegation were studied in different tissues. As we know, underreplication occurs during the formation of polytene chromosomes, however there may not be underreplication occurring in the somatic cells. Thus we can not definitely state that the underreplication of polytene chromosomes in the salivary glands is responsible for the white gene inactivation in ommatidial cells of adult flies. In order to make this association, both these phenomena should be analyzed in the same cell. Secondly, in the above experiments it is very difficult to define the cytological limits of the heterochromatin and euchromatin in the translocated chromosome fragment.

In 1981, Henikoff found that when the 87C heat shock puff locus was brought next to heterochromatin by a translocation, the translocated 87C region failed to puff or accumulate RNA in some nuclei, but puffed normally in other nuclei from the same individual. *In situ* hybridization experiments showed no detectable change in the polytenization of the inactivated gene site. From these experiments, he concluded that the mechanism of gene inactivation was the inability to be transcribed, rather than the somatic gene loss

and a consequent reduction in gene dosage associated with polytene chromosomes.

When the *rosy* locus was brought close to heterochromatin by an inversion, it also showed a variegated expression (Rushlow and Chovnick, 1984). Southern blotting experiments showed no detectable change in DNA content at the rosy locus and therefore lead to rejection of the mechanism that heterochromatic position effect results from underreplication of the position-affected gene. On the other hand, Northern blots gave direct evidence that position effect variegation is due to a defect in transcription (Rushlow *et al.*, 1984). That the gene inactivation is caused by a reduction in the accumulation of mRNA was also demonstrated for another two loci, *sgs-4* (Kornber and Kauffman, 1986) and *brown* (Henikoff and Dreesen, 1989).

All of these data lead to the conclusion that position effect variegation is the consequence of a change in the state of a gene rather than the number of its copies.

More recently, a molecular explanation has been proposed to account for the mechanism of position effect variegation and its spreading effect (Tartof *et al.*, 1984). When the euchromatic-heterochromatic junctions of w^{m4}, w^{mMc} and w^{m51b} were cloned, Tartof *et al.* (1984) found that the euchromatic breakpoints for these three variegating white mutations are clustered in a region located about 25 kb downstream of the *white* structural gene. Moreover, when irradiated by X-rays, a w^{m4} revertant was found which contained more than 3 kb of adjacent heterochromatic DNA. Based

on these results, they argued that the euchromatic-heterochromatic breakpoint is not sufficient to cause variegation. Instead they proposed that the DNA sequences that act as an initiation site for heterochromatic spreading are deeper in the heterochromatic domain. From the initiation site the spreading proceeds along the chromosome until it reaches some hypothetical termination site. They also proposed that there are many special non-histone chromosomal proteins, and perhaps covalently modified forms of histones as well, which frequently bind to the heterochromatic regions of the genome. Heterochromatin is formed by a cooperative interaction between DNA-protein or protein-protein in a polar manner from initiator to terminator. Position-effects are produced when a break occurs in heterochromatin between the initiator and terminator. The heterochromatin is propagated from the initiator site then extended into the euchromatic region until a similar terminator is reached.

4. Histones and Position Effect Variegation

Traditionally, all of the proteins associated with chromatin are divided into two general classes: histones and non-histone chromosomal proteins (NHCPs). Histones binding to DNA form the basic packing unit of chromatin referred to as the nucleosome. Histones occur in enormous quantities, and their total mass is about equal to that of the cell's DNA. Moreover, their relative amounts and stoichiometry with respect to DNA are nearly constant through out the eukaryotic kingdom. All of these facts suggest that histones play

an important part in the structure and function of chromatin (for review see Van Holde, 1989). If position effect variegation is caused by the spreading of heterochromatin into euchromatic genes to inactivate the affected genes, and since histones are the primary proteins involved in packing DNA into chromatin, histones may be involved in position effect variegation.

In most organisms, the histone genes are present in multiple copies (for review see Kedes, 1979). The histone genes in *Drosophila melanogaster* are located in region 39DE of the salivary gland polytene chromosomes according to *in situ* hybridization results (Pardue *et al.*, 1977 and Lifton *et al.*, 1978). Reassociation kinetics analyses showed that this sequences are repeated approximately 100 times per haploid genome.

Consistent with the suggestion that histones may play some role in position effect variegation, a deletion, which remove the histone gene complex located in the region 39DE2-3 to E1-2 on the left arm of second chromosome, can suppress position effect variegation in both *In(1) w^{m4}* and *$B^{sv}Y$* systems (Moore *et al.*, 1979; 1983), but a duplication of the histone gene complex did not enhance position effect variegation (Moore *et al.*, 1983). It appears that the decreasing of histone protein concentration may affect the packing of euchromatin into heterochromatin, but the increasing the amount of histone proteins does not have any effect on the spreading of heterochromatin showing that there are already enough histone genes present in the *Drosophila melanogaster* genome.

In 1980, Mottus *et al.* found that concentrations of n-butyrate and n-proprionate as low as 70 mM can dramatically suppress the variegated eye phenotype when $In(1)w^{m4}$ flies were treated with either these chemicals during the embryonic and larval stages. Since n-butyrate and n-proprionate can inhibit the histone deacetylase enzymes resulting in a rapid and extensive accumulation of hyperacetylated histones, particular H3 and H4 (Candido *et al.*, 1978), they argued that the effects of n-butyrate and n-proprionate on position effect variegation are due to the change in chemical states caused by the inhibition of histone deacetylas enzymes. Later in 1986, one dominant suppressor mutation of position effect variegation was found to show a lethal interaction with n-butyrate. It was suggested by Dorn *et al.* (1986) that this suppressor gene might affect histone deacetylation and probably encode an histone deacetylase enzyme.

On the other hand, it has been shown that heterochromatin and euchromatin contain approximately the same DNA/histone ratio (Berlowitz, 1965; Skinner and Ockey, 1971). Therefore, deletion of histone genes should affect both euchromatin and heterochromatin to the same degree, and should not have a direct effect on position effect variegation. It has been suggested by Michailidis *et al.* (1988) that decreased dosages of histone genes suppress position effect variegation probably by prolonging the development time of flies (Rushlow *et al.*, 1984). However, how the change of histone gene dosage and histone protein deacetylation affect position effect variegation is still not clear.

5. Genetic Loci that Modify Position Effect Variegation

Many single loci outside of the histone gene cluster can produce dominant mutations that modify (suppress and enhance) position-effect variegation. The first of these genetic modifers was described by Schultz (1950) and then extensively studied by Spofford (1967). But it was not until the later 70's and 80's that several papers were published on the isolation and characterization of these modifier genes (Henikoff, 1979; Reuter and Wolff, 1981; Reuter *et al.*, 1982, 1986; Sinclair *et al.*, 1983; 1989; Reuter and Szidonya, 1983; Locke *et al.*, 1988; Wustmann, *et al.*, 1989). The effects of modifier genes on position effect variegation are general. The mutations of these genes can affect different position effect variegation systems (Reuter *et al.*, 1982; Spofford 1967; Sinclair *et al.*, 1983; 1989; Locke *et al.*, 1988). There are probably between 30-100 modifier loci in the genome of *Drosophila melanogaster* (Locke *et al.*, 1988; Wustmann, *et al.*, 1989).

When studying a dominant position effect variegation ($In(3R)kar^{D}$), Henikoff (1979) found that a deletion of either of two very close regions (87EF and 87BC) on the third chromosome, which are less than 1% of the genome of *Drosophila melanogaster*, can suppress this dominant position effect variegation. Since he did not directly select for these events, he argued, by extrapolation, that modifier genes of position effect variegation must be very frequent and there are maybe hundreds of similar modifer loci located randomly in the Drosophila genome. By using a set of deficiences,

Reuter *et al.* (1987) mapped four dominant suppressors and one enhancer of position effect variegation in the region from 86C to 88B on the third chromosome of *Drosophila melanogaster.* Since this interval of about 155-160 bands does not contain an exceptional cluster of such genes, they also estimated by extrapolation that there are about 135 modifier loci of position effect variegation in the Drosophila genome. Wustmann *et al.* (1989) did cytogenetic fine structure studies of modifier genes of position effect variegation on chromosome arms 2L, 2R and 3R by using a total of 94 deficiencies and 83 duplications. By extrapolation, they argued strongly that there exist about 120 to 150 modifier genes in the *Drosophila melanogaster* genome. In contrast to the above estimations, Locke *et al.* (1988), after a survey of the literature and studying 12 P-element induced enhancers, suggested that there are probably no more than 20-30 suppressor/enhancer loci in the Drosophila genome.

By using EMS as mutagen, Sinclair *et al.* (1983) isolated 51 dominant suppressors and 3 dominant enhancers of position effect variegation in *Drosophila melanogaster.* Genetic mapping studies revealed that the Su(var) mutations fall into very discrete clusters. Twenty of 25 suppressor mutations mapped occupy four sites; one on the second chromosome and three on the third. Complementation analysis of Su(var) mutations located within the 2L cluster show that several different loci occupy this site. They suggested that these clusters may represent groupings of functionally related but distinct Su(var) loci. In 1986 when Reuter *et al.* did an extensive genetic analysis of 63 third chromosome suppressor mutations, only 12

different loci showing dominant suppression could be found, with no evidence for clustering of functionally related genes within each site.

From the above description, it seems that the number of modifier genes detected by mutagenesis (EMS, X-rays or P-elements) is much lower than the number detected by deficiency mapping. In order to explain the contradictory results, Reuter *et al.* (1987) suggested that for some loci only amorphic or strongly hypomorphic mutations might result in a detectable dominant suppressor and enhancer effect, respectively. Other loci might be repeated genes which could be only detected by deficiencies.

From the studies of four modifiers of position effect variegation and comparison with other modifier genes identified previously, Locke *et al.* (1988) divided these modifier genes into two classes. Class I genetic modifiers act as enhancers of position-effect variegation when they are duplicated but as suppressors when mutated or deficient. In contrast, class II genetic modifers enhance position-effect variegation when mutated or deleted but have a suppressing effect when duplicated. They further proposed a model, based on the law of mass action, to account for how such modifier genes function. They suggested that each class I modifier gene codes for a protein which is dosage dependent and required for the formation of heterochromatin. Thus mutated or deleted class I modifier genes can inhibit the formation and spreading of heterochromatin into a euchromatic region and therefore suppress position effect variegation. If these genes are duplicated, they can help the formation and spreading heterochromatin into euchromatic

regions and therefore enhance position effect variegation. In contrast, Class II modifiers may inhibit the class I gene products directly, promote euchromatin formation, or bind to hypothetical heterochromatin termination sites (see Tartof *et al.*, 1984). They further assumed that heterochromatin is formed by the assembly of a complex of multimeric proteins. Each multimeric proteins is composed of multiple copies (n) of subunits and each subunit is encoded by a class I gene. According to the law of mass action, at equilibrium the concentration of multimeric heterochromatin will be proportional to the n^{th} power of the concentration of each contributing subunits as expressed in the following equation:

$$K_{eq} = \frac{[(P_1P_2P_3P_4\cdots\cdots P_x)_n]}{[P_1]^n[P_2]^n[P_3]^n[P_4]^n\cdots\cdots[P_x]^n}$$

(where P represents each of the different protein subunits and n is the number of each subunits present in each heterochromatin unit). Thus an increase in the gene dosage of any one subunit gene would drive the equilibrium toward the formation of heterochromatin (resulting in enhanced variegation) and a decreased gene dosage would pull the equilibrium away from heterochromatin (resulting in suppressed variegation), both in an exponential way.

Consistent with this model in the last paragraph, several non-histone proteins have been shown to exhibit preference for binding to heterochromatin (Levinger and Varshavsky, 1982; James and Elgin, 1986). The cDNA encoding a specific heterochromatin binding protein called HP-1 has been cloned by Eissenberg *et al.* (1987), and this HP-1 gene has been located to a dominant modifier locus (Su

(var) 205) of position effect variegation at 29A on the polytene chromosome. Eissenberg *et al.* (1990) reported that the Su(var)205 mutation is a point mutation of the HP-1 gene, causing aberrant splicing of the gene transcript.

By using a chromosomal walking experimental approach, one of the modifier genes (Suvar(3)7), located within 87E region of polytene chromosome, has been cloned and extensively studied by Reuter *et al.* (1990). When the cloned gene was reintroduced into the Drosophila genome by P-element mediated transformation, they found that increasing the copies to three or more can enhance variegation progressively corresponding to gene dose, whereas reducing the copies of this gene from two to one suppressed the position effect variegation. The sequence of the cDNA of this gene predicted a 923-amino acid protein. This deduced protein show five potential Zinc fingers of the Cys_2-His_2 type, in which the five potential zinc-fingers are separated by 40 to 107 residues. This is very different from other reported Zinc-finger proteins. It has been suggested (Reuter *et al.*, 1990) that the large spacing of fingers in Suvar(3)7 might provide a good way for this protein to bind to relatively distant sites on DNA for the formation of heterochromatin.

6. P-elements in Mutagenesis and Transposon Tagging

Mainly, three kinds of mutagenesis methods (ionizing and ultraviolet radiations, chemical mutagens and P-M mutagenesis) have been used to induce mutations in *Drosophila melanogaster* (Ashburner, 1989a; Grigliatti, 1986). Although each method has its

own advantages and disadvantages, P-M mutagenesis is preferred over the other two methods. First, it provides an efficient method for transposon tagging and cloning since the transposition of P-element can induce high frequency of mutation (insertions and deletions) in many genomic regions. A P-element can then be used as a probe to find the mutated genes (Grigliatti, 1986). Second, the transposition of P-elements is under genetic control and can be readily carried out by mating suitable fly stocks.

A large number of transposable element families have been found to exist in the *Drosophila melanogaster* genome, including copia-like elements, foldback elements and P-elements. (Ashburner, 1989b; Grigliatti, 1986 for reviews). To date P-elements are the best studied transposable element family. They can produce a group of unusual genetic traits called hybrid dysgenesis when they transpose. This feature is very useful for cloning the genes by transposon-tagging and many other types of applications (Kidwell, 1986).

Hybrid dysgenesis is "a syndrome of correlated genetic traits that is spontaneously induced in hybrids between certain mutually interacting strains, usually in one direction only" (Sved, 1976). Two independent systems of hybrid dysgenesis have been identified, that is I-R (for inducer and reaction) and P-M (for paternal and maternal). The genetic traits associated with P-M hybrid dysgenesis include male recombination, sterility, chromosomal rearrangements and various kinds of unstable mutations. P-M hybrid dysgenesis is due to the transposition of transposable P-elements. There are generally two classes of P-elements, autonomous and defective.

Autonomous P-elements are about 2.9 kb long and appear to encode a transposase which is required for the transposition of P-elements. Defective P-elements are usually derived from autonomous P-elements by internal deletions. They can not produce functional transposase, but can be mobilized by the transposase produced from intact P-elements in the same genome.

P-elements are present, usually in multiple copies, in P strains of *D. melanogaster*, but not in M strains. In addition, the transposition of P-elements is under genetic control. They can only transpose in a germ line state called M cytotype, but they are relatively stable in somatic cells or in a germ line state called the P cytotype. Cytotype is inherited maternally. Thus, transposition and hybrid dysgenesis can occur only when potentially active P elements are introduced to a M cytotype contributed by the female parent. Transposition and hybrid dysgenesis is not induced in the reciprocal cross since, in this case, the female transmits the P cytotype (for a review see Grigliatti, 1986).

Using the knowledge of the molecular basis of P-M hybrid dysgenesis, a very powerful technique, called P-element tagging, has been developed for cloning genes in *D. melanogaster*. Many mutations, especially dysgenically induced ones, can be caused by the insertion of a P-element in or near the gene of interest. A P element probe can be used to recover the flanking sequences at the insertional sites. This powerful technique has been used to clone many genes (Kidwell, 1986). However, a typical wild P-strain contains 30-50 P-element sequences dispersed throughout the

genome (Laski *et al.*, 1986). This distribution of P-elements greatly limits the utility of this approach since the dozens of P-elements present in the stock make it very difficult to identify and clone the specific P-element responsible for the mutant of interest. Furthermore, newly induced insertional mutations are highly unstable and are frequently lost, unless they are stabilized in a P cytotype by crossing back to a P strain.

These problems can be overcome by using an *in vitro* constructed P-element insertion (pUChsneo) in combination with a single stable source of transposase (Δ2-3(99B)system). Steller and Pirrotta (1985) constructed a P transposon called pUChsneo (see Figure 1) which contains the *E coli* neomycin resistance gene and the entire bacterial plasmid vector pUC8 flanked by the P element inverted terminal repeats. This transposon can insert into a genomic site and induce a mutation. Since it carries the plasmid replicon, it can then be used for plasmid rescue of flanking genomic sequences (Perucho *et al.*, 1980). The transposon pUChsneo present is relatively stable in the genome since it can not produce transposase, but can be efficiently mobilized by a different P-element that encodes transposase, but can not transpose itself. In my experiments, I used a transposase-producing P-element described by Robertson *et al.* (1988), called Δ2-3(99B). By making a cross between flies containing pUChsneo P-transposon and flies bearing a Δ2-3(99B) P-transposon, these two P-tranposons can be brought into the same genome. Consequently, the pUChsneo P-element can be mobilized by the transposase function provided by the Δ2-3(99B) P-transposon. After

it inserts into a new site, the transposase source can be genetically crossed out.

If the site of insertion is within a class I modifer gene a dominant suppressor mutation. will result. I used the stable transposase-producing Δ2-3 P-element to mobilze the pUChsneo P-element and induce insertions causing mutations that suppress position effect variegation. After crossing out the Δ2-3 P-element the suppressor mutants should be stable and can be used to recover the gene via plasmid rescue (Cooley *et al.*, 1988).

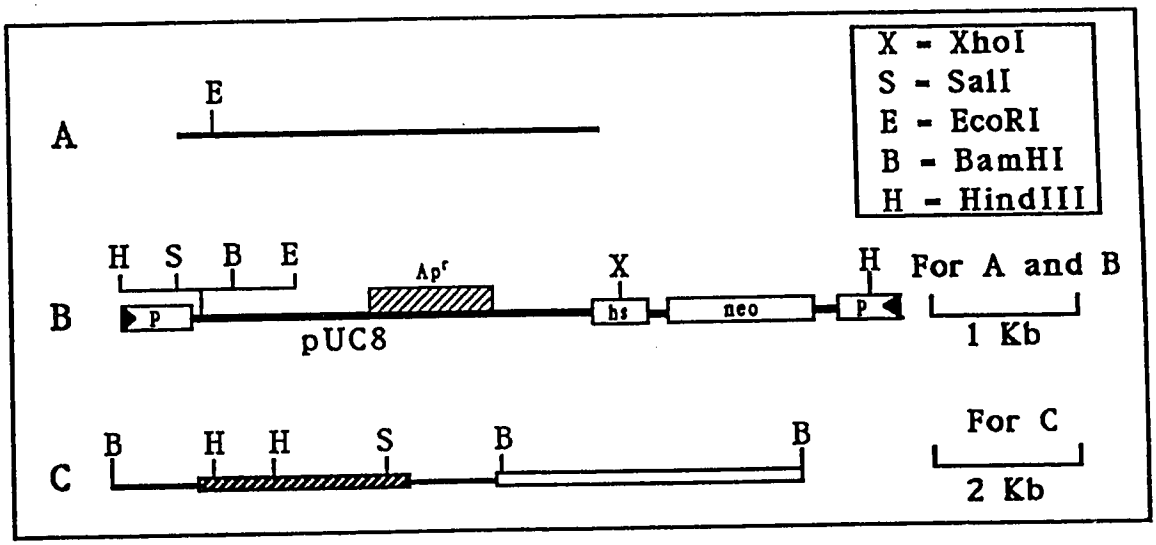


Figure 1. Restriction maps of plasmids. The restriction sites are shown above the map.

(A) The restriction map of plasmid rescued from the double digestion (SalI and XhoI) of P[pUChsneo](9C), mwh red e (#26) genomic DNA.

(B) The restriction map of pUChsneo P-element adapted from Steller and Pirrotta (1985). hs is hsp70 heat-shock promoter, neo is the neomycin resistence gene and P represents inverted P-element ends). ▨ is the position of ampicilin resistence gene of pUC8.

(C) The restriction map of Pπ25.1 adapted from Spradling and Rubin (1982). ▬ represents the 2.9 Kb P-element. ▭ is the pBR322 vector sequences. ——— represents flanking Drosophila genomic DNA sequences from 17C region of polytene chromosome. The *Bam*HI-*Hin*d III fragments of P-element were used for probing the genomic Southern blots of suppressor mutations.

II. Materials and Methods

1. Description of *Drosophila melanogaster* Stocks

All *Drosophila melanogaster* stocks used in my reserach are described in Table 1. A detailed description of the phenotypes of the different mutations and balancer chromosomes can be found in Lindsley and Grell (1968). A description of the flies bearing the pUChsneo P-element is in Pirrotta (1986) while Δ2-3 (99B) P-element is described in Robertson *et al.* (1988). For more information about the phenotype and cytogenetic properties of $In(1)w^{m4}$, w^{m4} see Tartof *et al.* (1984). The $Inscy/Inscy/B^{sv}\ Y\ y^{+}$ stock is described in Brosseau (1964).

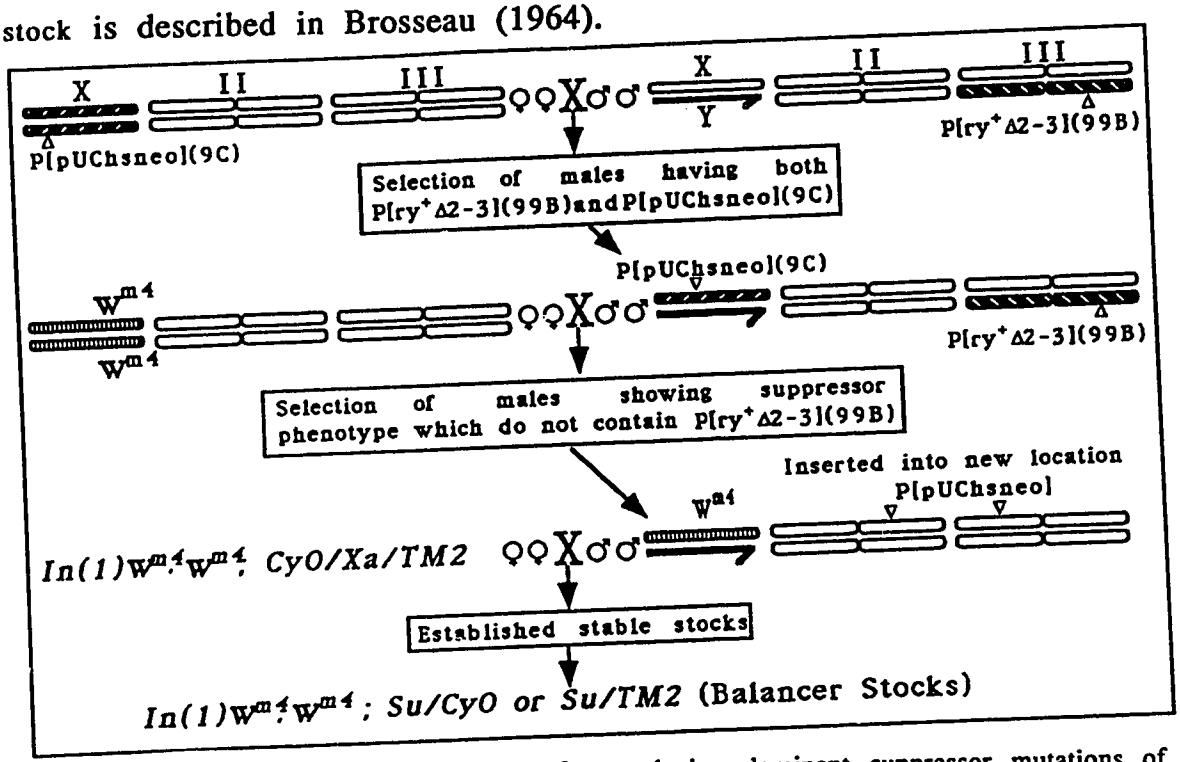


Figure 2. General genetic cross scheme for producing dominant suppressor mutations of w^{m4} variegation by P-element mutagenesis. By crossing virgin females having pUChsneo P-element on their X-chromosomes to males bearing Δ2-3 transposase-producing P-element on their third chromosome, males containing both Δ2-3 and pUChsneo P-elements were selected. These males were then mated to w^{m4} virgin females. From the predominantly red-white mottled eye male progeny, males with red eyes, each bearing a putative suppressor mutation, were selected, and then crossed to virgin females with genotype w^{m4}; *CyO/Xa/TM2* (#58), from which an appropriately balanced stable stock was obtained eventually.

Table 1

Description of *Drosophila melanogaster* stocks used in my research

Lab Stock #	Genotype	P or M Strain	Sources
4	*In(1)w^{m4}, w^{m4}; T (2;3)ap^{Xa}/TM3, Sb e^{s} p^{p}*	M	K. D. Tartof[a]
8	*In(1)w^{m4}, w^{m4}; MRS/TM6, Ubx e*	M	K. D. Tartof
16	*XX,w^{48h}/XY*	M	K. D. Tartof
26	*P[pUChsneo](9C); mwh red e*	neo-P	M. Russell[b]
27	*w; Sb e P[ry^{+} Δ2-3](99B)/TM6, Ubx e*	Δ2–3 P	M. Russell
30	*Gl Sb H / Payne*	M	Bowling Green[c]
35	*π2 wild-type*	P	Bowling Green
36	*Canton-S wild-type*	M	Bowling Green
37	*Oregon-R wild-type*	M	Bowling Green
39	*ry^{506} P[ry^{+}Δ2-3](99B)*	Δ2–3 P	Bowling Green
40	*CyO/Sp; ry^{506} Sb P[ry^{+}Δ2-3](99B)*	Δ2–3 P	Bowling Green
58	*In(1)w^{m4}, w^{m4}; CyO/T(2;3)ap^{Xa}/TM2, Ubx e*	M	J. Locke[d]
62	*In(1)w^{m4}, w^{m4}; CyO/T(2;3)ap^{Xa}*	P	J. Locke
67	*In(1)w^{m4}, w^{m4}; +/+*	M	J. Locke
94	*In(1)w^{m4}, w^{m4}; al dp b pr sp/CyO*	M	J. Locke
228	*Inscy/Inscy/B^{sv} Y y^{+}*	M	D. Sinclair[e]

Notes:

a. These stocks were obtained from Dr. K. D. Tartof, Institute for Cancer Research, Fox Chase Cancer, Philadephia, Pennsylvania 19111, U.S.A.

b. These Stocks were kindly provided by Dr. M. Russell, Department of Genetics, University of Alberta, Edmonton, Alberta, T6G 2E9.

c. These stocks were received from Bowling Green State University, Mid-America Drosophila Stock Center.

d. These stocks were synthesized in Dr. J. Locke's Lab.

e. This stock was obtained from Dr. D. Sinclair, Department of Zoology, University of British Columbia, Vancouver, British Columbia, U6T 2A9.

2. Media and Culture Conditions of Drosophila Stocks

All *Drosophila melanogaster* stocks were grown on a standard medium at room temperature (about 22°C) in small standard vials (2.4 X 9.4 cm). To expand the fly culture, the flies were grown in milk bottles. When making crosses, generally about 10 flies were put into a vial and about 40 flies were put in a bottle.

Standard Medium is made up of the following ingredients: 10 g Agar, 100 g Sucrose, 100 g Brewer's yeast, 100 ml Chloramphenicol

(1 g/L stock solution), 10 ml Proprionic acid, 4.3 g NaH_2PO_4 and 2.7 g Na_2HPO_4. Add water to 1 liter.

3. Genetic Screens for Suppressor Mutations

In my experiments screening for dominant suppressors of PEV, a two generation cross-scheme was used to generate and identify the mutations. The general cross scheme is as illustrated in Figure 2. In the first generation cross, about 30 virgin females of the genotype *P[pUChsneo](9C); mwh red e* (#26), having a pUChsneo P-element located on 9C of the X chromosome were crossed to about 30 males of genotype *w; Sb e P[ry+Δ2-3](99B)/TM6* (#27) or ry^{506} *P[ry+ Δ2-3](*99B) (#39) in each bottle (The numbers after each genotype indicate the stock number in Dr. John Locke's stock list). From the F_1 the potential dysgenic males, having both the *P[pUChsneo](9C); mwh red e* and *P[ry+Δ2-3](99B)* chromosomes, were selected and crossed out to virgin females of *In(1)*w^{m4}, w^{m4}*; MRS/TM6, Ubx e* (#8) or *In(1)*w^{m4}, w^{m4}*; CyO/Xa/TM2, Ubx e* (#58) genotype to produce progeny that were screened for suppressor mutations. Each experiment usually contained 16 bottles with about 15 dysgenic males and 30 virgin females per bottle.

Four different cross schemes (A, B, C, D, See Figure 3, 4, 5, 6) were used at the begining of the screening experiments. I chose to concentrate on cross scheme D for the following reasons.

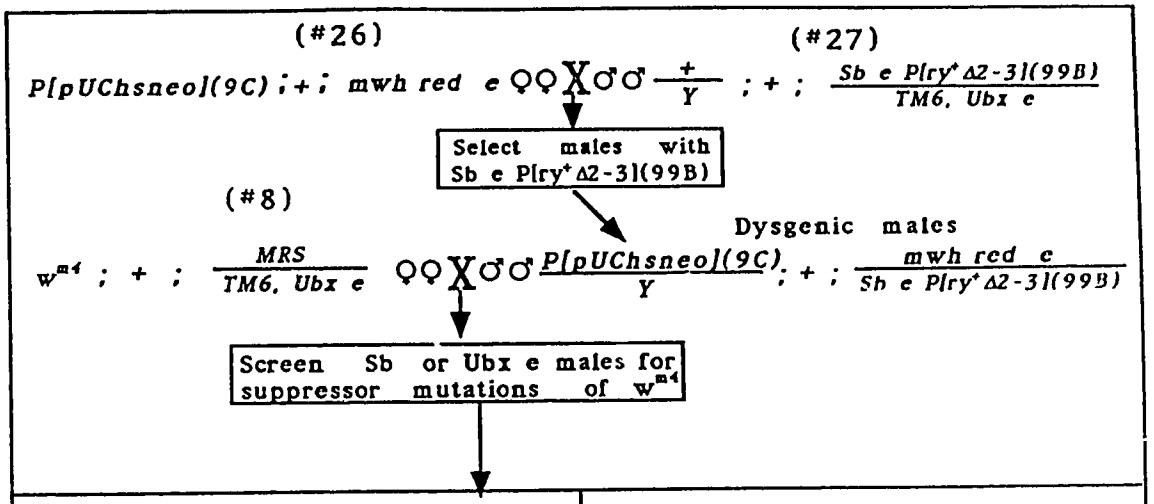
A) Both *w; Sb e P[ry+Δ2-3](99B)/TM6* (#27) and ry^{506} *P[ry+ Δ2-3](99B)* (#39) have Δ2-3 P-element located on the third chromosome that produces transposase to mobilize the pUChsneo P-element and induce mutations. During my initial experiments, I

found that *ry^{506} P[ry+ Δ2-3](99B)* (#39) appeared to have a much stronger transposase ability than that of *w; Sb e P[ry+Δ2-3](99B)/TM6* (#27). When using *w; Sb e P[ry+Δ2-3](99B)/TM6* (#27), no suppressor mutations were obtained from three trays of crosses. However, some suppressor mutations were obtained when *ry^{506} P[ry+ Δ2-3](99B)* (#39) was used (see Table 2). Additionally, *w; Sb e P[ry+Δ2-3](99B)/TM6* (#27) is heterozygous for the P[ry+Δ2-3](99B) P-element. Therefore after the first generation only half of the male progeny contained both pUChsneo P-element and could be used for further crosses. However, *ry^{506} P[ry+ Δ2-3](99B)* (#39) is homozygous for Δ2-3(99B) P-element and all the males could be used for the next cross. Taking these two aspects into consideration, I chose *ry^{506} P[ry+ Δ2-3](99B)* (#39) as the P[ry+Δ2-3](99B) P-element source for further screening experiments.

B) In the begining, *In(1)w^{m4}, w^{m4}; MRS/TM6, Ubx e* (#8) flies were used as the second generation cross virgin females. Subsequently I found that *In(1)w^{m4},w^{m4}; CyO/Xa/TM2, Ubx e* (#58) had some advantages over *In(1)w^{m4}, w^{m4}; MRS/TM6, Ubx e* (#8). First, once putative suppressor mutations were obtained from screening, it was much easier for me to confirm the suppressor mutations and establish stocks when *In(1)w^{m4},w^{m4}; CyO/Xa/TM2, Ubx e* (#58) flies were used (see Figure 6). In contrast, when *In(1)w^{m4}, w^{m4}; MRS/TM6, Ubx e* (#8) flies were used, further confirmation of suppressor mutations was very difficult because the crosses with putative suppressor males (necessary for establishing and confirmation of suppressors) produced many kinds of progeny. It was hard to distinguish some progeny from each other. Second,

In(1)w^{m4}, w^{m4}; MRS/TM6, Ubx e (#8) flies appeared to have a higher frequency of Y-chromosome non-disjunction. This produces an extra Y-chromosome that can suppress position effect variegation, thereby causing a problem in screening for suppressor mutations by creating false positives. Third, *In(1)w^{m4},w^{m4}; CyO/Xa/TM2, Ubx e* (#58) flies grow better than *In(1)w^{m4}, w^{m4}; MRS/TM6, Ubx e* (#8) in our lab, thereby making large screens easier. Fourth, when *In(1)w^{m4},w^{m4}; CyO/Xa/TM2, Ubx e* (#58) flies were used, two phenotypically distinct types of progeny were produced. The progeny of a non-virgin *In(1)w^{m4},w^{m4}; CyO/Xa/TM2, Ubx e* (#58) will not affect the screening results. However, with *In(1)w^{m4}, w^{m4}; MRS/TM6, Ubx e* (#8) progeny and parents could be confused.

For these reasons, I decided to concentrate on cross scheme D (see Figure 6). This cross scheme used stocks *ry^{506} P(ry+ Δ2-3)(99B)* (#39), *P[pUChsneo](9C); mwh red e* (#26) and *In(1)w^{m4},w^{m4}; CyO/Xa/TM2, Ubx e* (#58) to start the cross scheme. In the second generation, from predominantly mottled red-white-eye male progeny, the males with almost wild-type eyes were selected since they bear the putative suppressor mutations. Putative suppressor males Cy Ubx e which do not contain P[ry+Δ2-3](99B) P-element were selected. These males were crossed to *In(1)w^{m4},w^{m4}; CyO/Xa/TM2, Ubx e* (#58) females. At the same time, the putative suppressor males with Xa were also selected, but from the second generation crosses, I could not distinguish two kinds of Xa flies, one containing *mwh red e* chromosome and the other having the *ry^{506} P[ry+Δ2-3](99B)* chromosome. However by recrossing these Xa males to *In(1)w^{m4},w^{m4};* CyO/Xa/TM2, Ubx e (#58) females, they can be



Genotype	Phenotype
$\frac{w^{m4}}{Y}$; $\frac{+^*}{+}$; $\frac{mwh\ red\ e^*}{MRS}$	Sb
$\frac{w^{m4}}{Y}$; $\frac{+^*}{+}$; $\frac{mwh\ red\ e^*}{TM6,\ Ubx\ e}$	Ubx e
$\frac{w^{m4}}{Y}$; $\frac{+^*}{+}$; $\frac{Sb\ e\ P[ry^+\Delta 2\text{-}3](99B)}{TM6,\ Ubx\ e}$	Sb Ubx e
$\frac{w^{m4}}{Y}$; $\frac{+^*}{+}$; $\frac{Sb\ e\ P[ry^+\Delta 2\text{-}3](99B)}{MRS}$	Dead

Figure 3. Cross scheme A for the screening of suppressor mutations of w^{m4}. Lab stock numbers are shown above the genotype when appropriate. Asterisk (*) is the location of putative suppressor mutations supposed to be caused by the insertion of a pUChsneo P-element. P[pUChsneo](9C) represents the X chromosome having a pUChsneo P-element (see Steller and Pirrotta, 1985); w^{m4} represents $In(1)w^{m4}$, w^{m4}. P[ry+Δ2-3](99B) is Δ 2-3 transposase-producing P-element inserted into 99B region of the third chromosome (see Robertson et al., 1988). An explanation of this cross scheme is given in the text of Materials and Methods section 3.

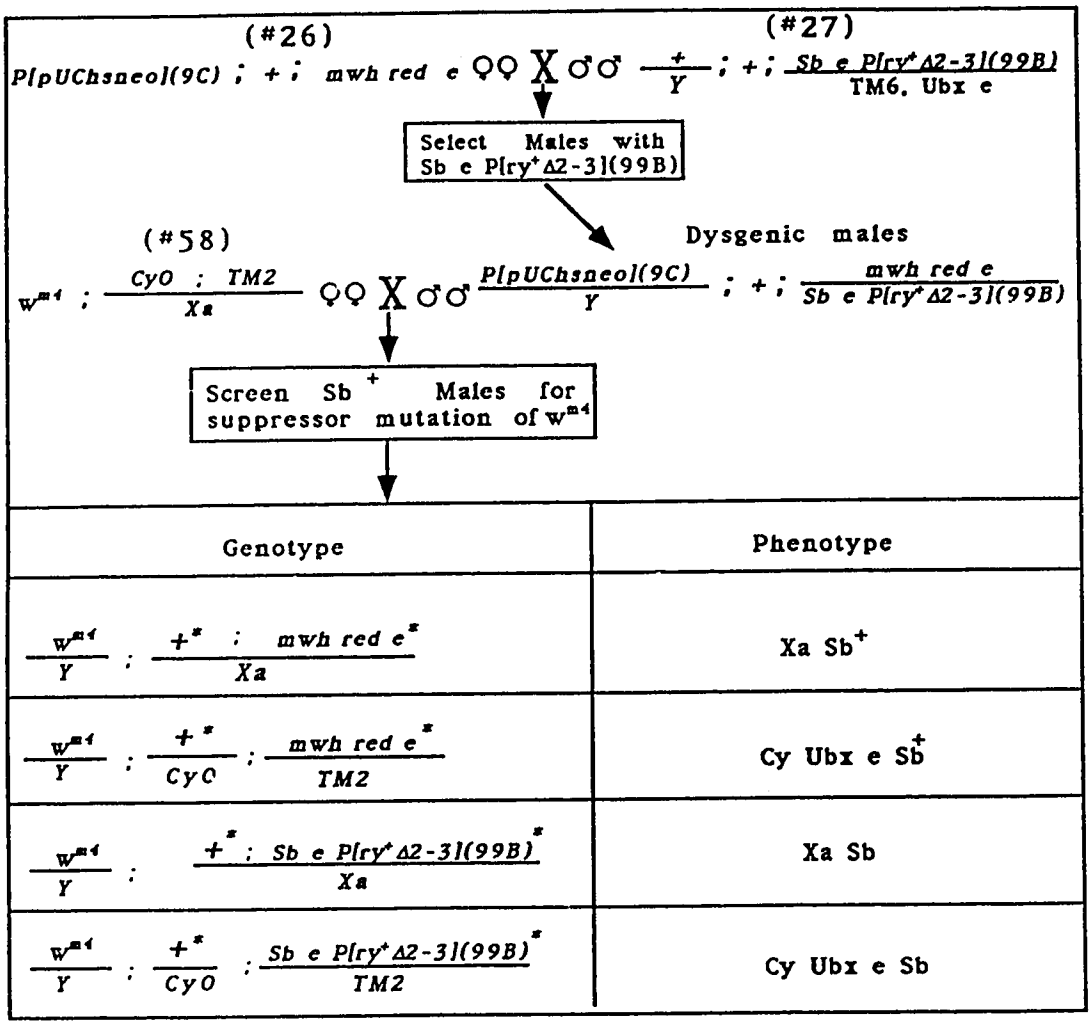
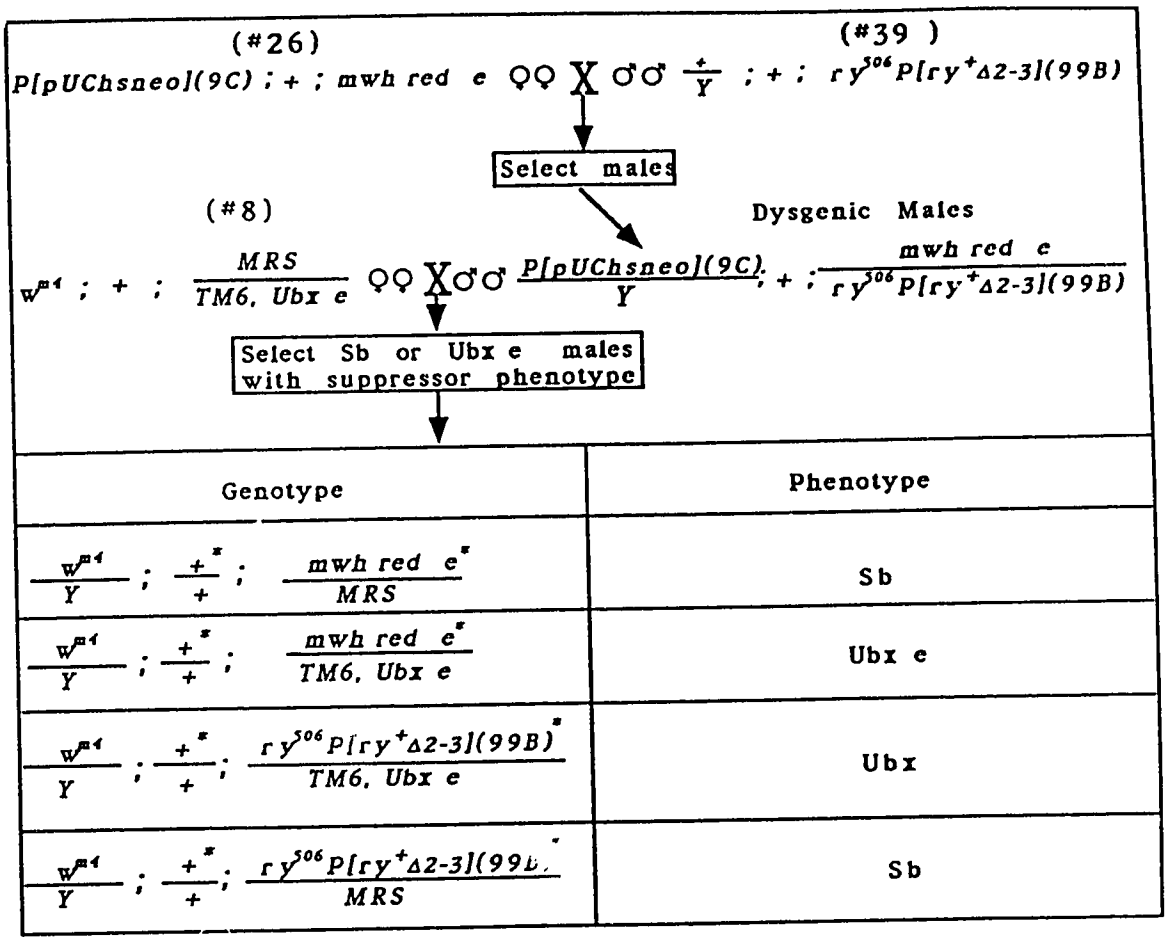
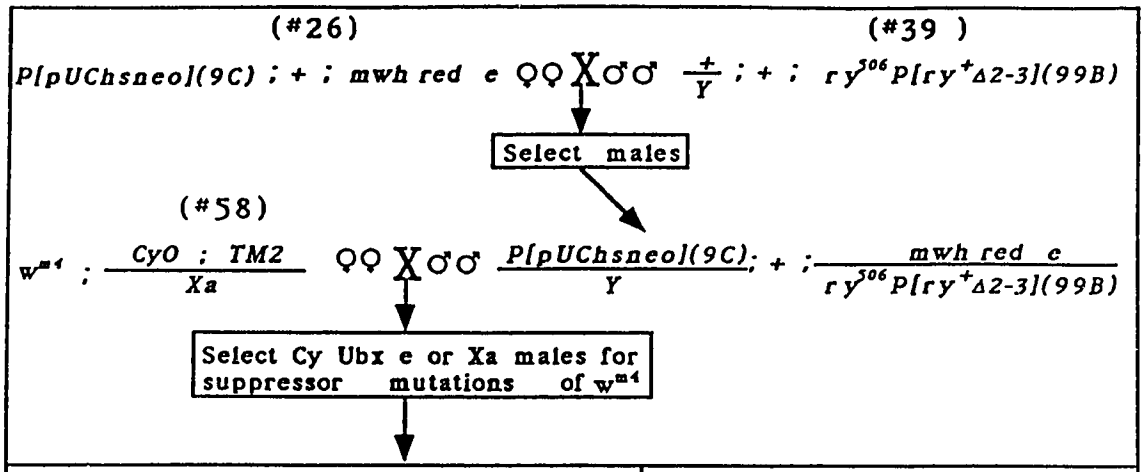


Figure 4. Cross scheme B for the screening of suppressor mutations of w^{m4}. Lab stock numbers are shown above the genotype when appropriate. Asterisk (*) is the location of putative suppressor mutations supposed to be caused by the insertion of a pUChsneo P-element. P[pUChsneo](9C) represents the X chromosome having a pUChsneo P-element (see Steller and Pirrotta, 1985); w^{m4} represents $In(1)w^{m4}$, w^{m4}. P[ry+Δ2-3](99B) is Δ 2-3 transposase-producing P-element inserted into 99B region of the third chromosome (see Robertson et al., 1988). An explanation of this cross scheme is given in the text of Materials and Methods section 3.



Genotype	Phenotype
$\frac{w^{m4}}{Y}$; $\frac{+^{*}}{+}$; $\frac{mwh\ red\ e^{*}}{MRS}$	Sb
$\frac{w^{m4}}{Y}$; $\frac{+^{*}}{+}$; $\frac{mwh\ red\ e^{*}}{TM6,\ Ubx\ e}$	Ubx e
$\frac{w^{m4}}{Y}$; $\frac{+^{*}}{+}$; $\frac{ry^{506}P[ry^{+}\Delta 2\text{-}3](99B)^{*}}{TM6,\ Ubx\ e}$	Ubx
$\frac{w^{m4}}{Y}$; $\frac{+^{*}}{+}$; $\frac{ry^{506}P[ry^{+}\Delta 2\text{-}3](99B)^{*}}{MRS}$	Sb

Figure 5. Cross scheme C for the screening of suppressor mutations of w^{m4}. Lab stock numbers are shown above the genotype when appropriate. Asterisk (*) is the location of putative suppressor mutations supposed to be caused by the insertion of a pUChsneo P-element. P[pUChsneo](9C) is the X chromosome having a pUChsneo P-element (see Steller and Pirrotta, 1985). w^{m4} represents $In(1)w^{m4}$, w^{m4}; and in ry506 P[ry+ Δ2-3](99B) there is a Δ 2-3 transposase-producing P-element (see Robertson et al., 1988). An explanation of this scheme is given in the text of Materials and Methods section 3.



Genotype	Phenotype
$\frac{w^{m4}}{Y} ; \frac{+^{*}}{Xa} ; \frac{mwh\ red\ e^{*}}{}$	Xa
$\frac{w^{m4}}{Y} ; \frac{+^{*}}{CyO} ; \frac{mwh\ red\ e^{*}}{TM2,\ Ubx\ e}$	Cy, Ubx e
$\frac{w^{m4}}{Y} ; \frac{+^{*} ; ry^{506}P[ry^{+}\Delta 2\text{-}3](99B)^{*}}{Xa}$	Xa
$\frac{w^{m4}}{Y} ; \frac{+^{*}}{CyO} ; \frac{ry^{506}P[ry^{+}\Delta 2\text{-}3](99B)^{*}}{TM2,\ Ubx\ e}$	Cy, Ubx

Figure 6. Cross scheme D for the screening of suppressor mutations of w^{m4}. Lab stock numbers are shown above the genotype when appropriate. Asterisk (*) is the location of putative suppressor mutations supposed to be caused by the insertion of a pUChsneo P-element. P[pUChsneo](9C) is the X chromosome having a pUChsneo P-element (see Steller and Pirrotta, 1985). w^{m4} represents $In(1)w^{m4}$, w^{m4}; and in ry506 P[ry+ Δ2-3](99B) there is a Δ 2-3 transposase-producing P-element (see Robertson et al., 1988). An explanation of this scheme is given in the text of Materials and Methods section 3.

distinguished since flies with *mwh red e* chromosome express recessive e. Once they were recrossed to $In(1)w^{m4},w^{m4}$; *CyO/Xa/TM2, Ubx e* (#58) virgin females, the progeny having e phenotype could be selected (see Figures 7 and 8).

4. Establishment of Suppressor Mutation Stocks

Once a putative suppressor mutation male was obtained from screening, it was crossed to about 5 virgin females with the genotype $In(1)w^{m4},w^{m4}$; *CyO/Xa/TM2, Ubx e* (#58) (see Figures 7 and 8). By examining the progeny, it is easy to confirm and assign any suppressor mutation to the second or third chromosome.

From both crosses (see Figures 7 and 8), we can see that if the putative suppressor is transmissible, all Cy Ubx e flies should be suppressed and all Cy Xa Ubx flies should not be suppressed, regardless of where the suppressor mutations are located.

If the suppressor mutation is located on the second chromosome, the suppressors should segregate from CyO, but be independent of the *mwh red e* and *TM2, Ubx e* chromosomes. Therefore, all Xa and Xa Ubx flies should be suppressed and both CyO Xa and CyO Xa Ubx flies should not be suppressed. If the suppressor mutation is located on the third chromosome, it would segregate from *TM2, Ubx e*, but be independent of the *CyO* or *Xa* chromosomes. Therefore, all the Xa and Xa CyO Ubx$^+$ flies should be suppressed and all the Xa Ubx and CyO Ubx Xa should not be suppressed. If a putative suppressor mutation did not fit into these categories, it was discarded. These putative suppressor mutations were non-transmitted and possibly due to: (1) an extra Y-chromosome, (2)

fluctuation of expression of the white gene in w^{m4} flies, or (3) suppressor mutations located on the fourth chromosome.

The screening procedure used would preclude isolating any X linked suppressor mutations. Furthermore none of the suppressor mutations that I found were located on the Y chromosome. If they were located on the Y chromosome, all the males would be suppressed and all the females would not. I did not find this case with any putative suppressor mutations.

Since all the Cy Ubx e flies contain suppressor mutations if the mutation is transmissible, I could just simply pick males and virgin females of Cy Ubx e flies and sib-mate them to establish a stock.

If a suppressor mutation is located on the second chromosome and is lethal or sterile as a homozygote, the following flies and ratios from the sib-mating will be observed:

w^{m4}; +/CyO; mwh red e* 1

w^{m4}; +/CyO; mwh red e/TM2, Ubx e* 2

In such case Ubx^{+} flies were selected and used to make sib-crosses. The genotype of stable stocks would be *w^{m4};+*/CyO; mwh red e*. If the suppressors are located on the second chromosome but homozygous viable and fertile, the final stable stock should be *w^{m4}; +*/+*; mwh red e.*

Similarly if a suppressor is located on the third chromosome, the homozygous viable and fertile stocks should be *w^{m4}; +/+; mwh red e**, and if sterile or lethal as homozygotes the stocks will be *w^{m4}; +/+; mwh red e*/TM2, Ubx e.*

When crosses between some of the putative suppressor mutation flies and *In(1)w^{m4}; CyO/Xa/TM2, Ubx e* (#58) were made, I

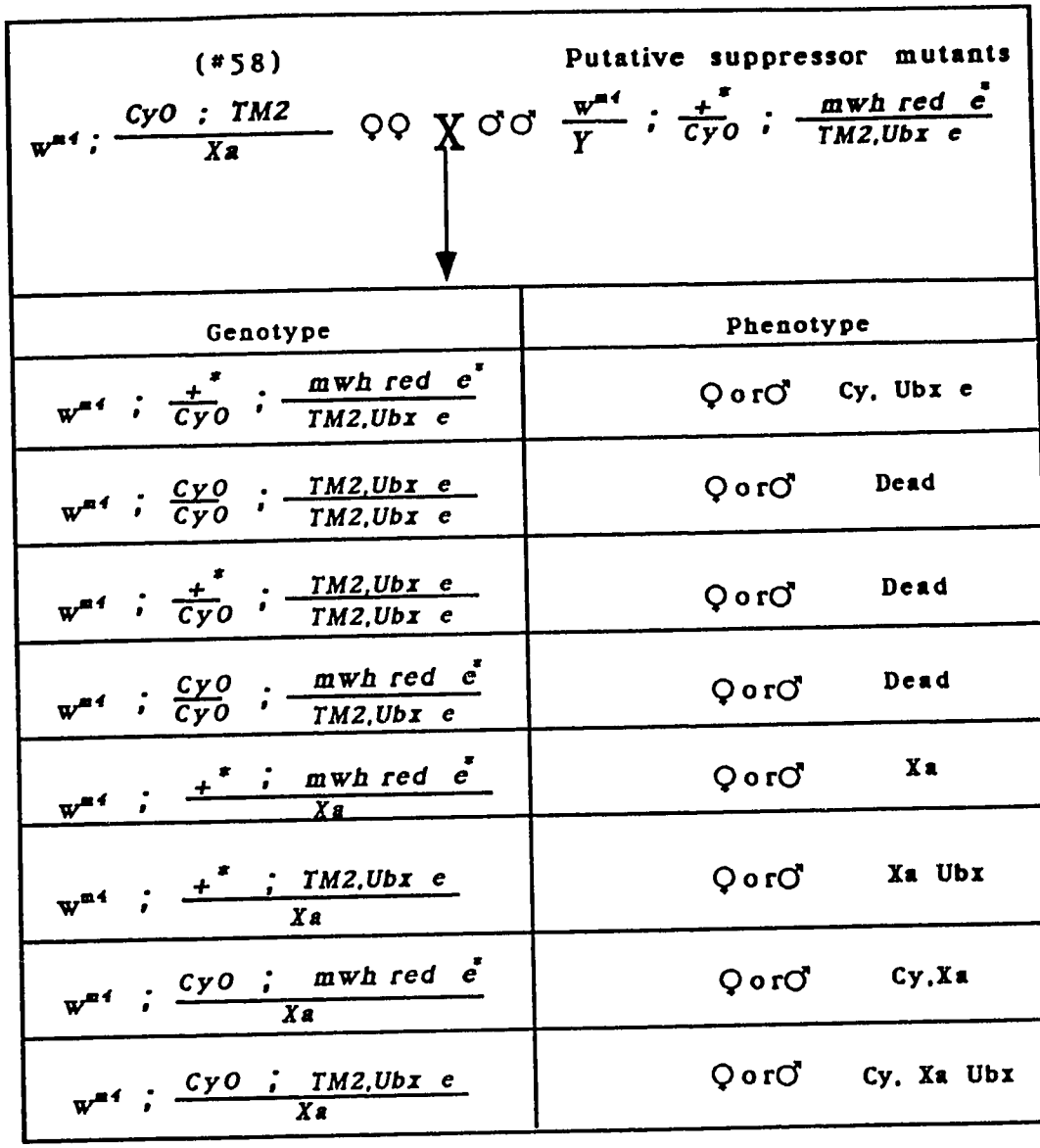


Figure 7. Cross for the confirmation and assignment of putative suppressor mutations to the second or third chromosome. Lab stock numbers are shown above the genotype when appropriate. An explanation of this cross is in the Materials and Methods section 4. Asterisk (*) is the location of putative suppressor mutations supposed to be caused by the insertion of a pUChsneo P-element. w^{m4} represents $In(1)w^{m4}$, w^{m4}.

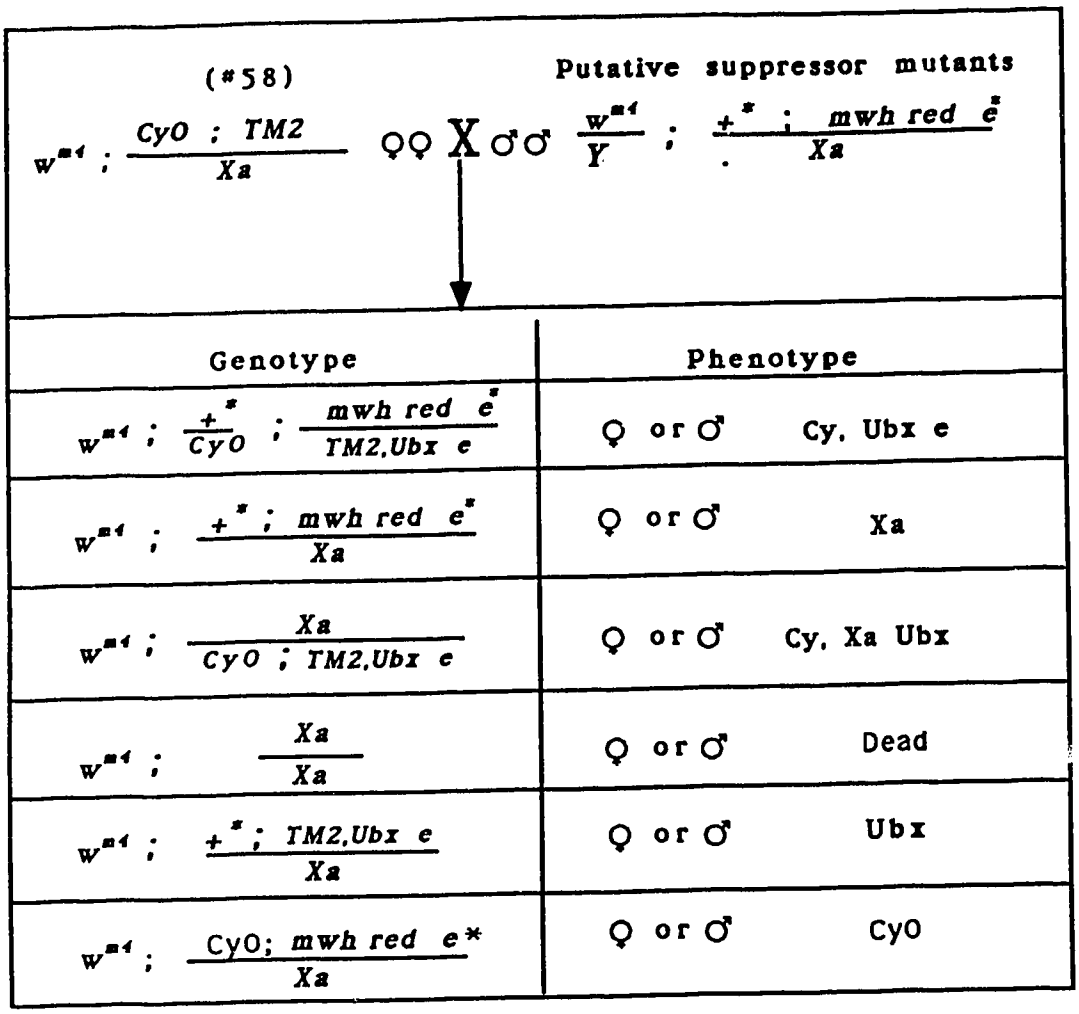


Figure 8. Cross for the confirmation and assignment of putative suppressor mutations to the second or third chromosome. Lab stock numbers are shown above the genotype when appropriate. An explanation of this cross is in the Materials and Methods section 4. Asterisk (*) is the location of putative suppressor mutations supposed to be caused by the insertion of a pUChsneo P-element. w^{m4} represents $In(1)w^{m4}$, w^{m4}.

found that they were lethal over either the CyO or the TM2 balancer chromosome since no Cy Ubx e flies were found from these crosses (see figure 7 and 8). Also from these crosses, it could be determined that all of these suppressor mutations were located on the third chromosome. To make stocks, the suppressor Xa males were crossed to w^{m4}; *Xa/TM3, Sb e* (#4) virgin females. Then the progeny were sib-mated and the suppressor mutations were balanced by TM3.

5. Suppression Caused by an Extra Y-chromosome

Since an additional Y-chromosome can suppress position effect variegation, my putative suppressor mutations might be due to an extra Y-chromosome. To eliminate this possibility, crosses between suppressor mutation males and attached *XX*, w^{48h} (#16) virgin females were made (see Figure 9). In this cross, if the suppression is not due to an extra Y-chromosome, only two kind of flies are produced. Males ($w^{m4}/0$) are sterile and females (XXY) are fertile, therefore, these progeny can not produce generation III. If the suppression is caused by an additional Y-chromosome, another kind of fertile male (w^{m4}/Y) flies would be produced, so the progeny of the above cross should be fertile.

To test the fertility of the progeny from the above cross, several flies of each sex were put into vials, and then observed for about 10 days to see if they could produce any progeny. If no progeny were produced, the suppression was assumed not to be due to an extra Y-chromosome. If progeny were produced, the suppression was probably caused by an extra Y-chromosome.

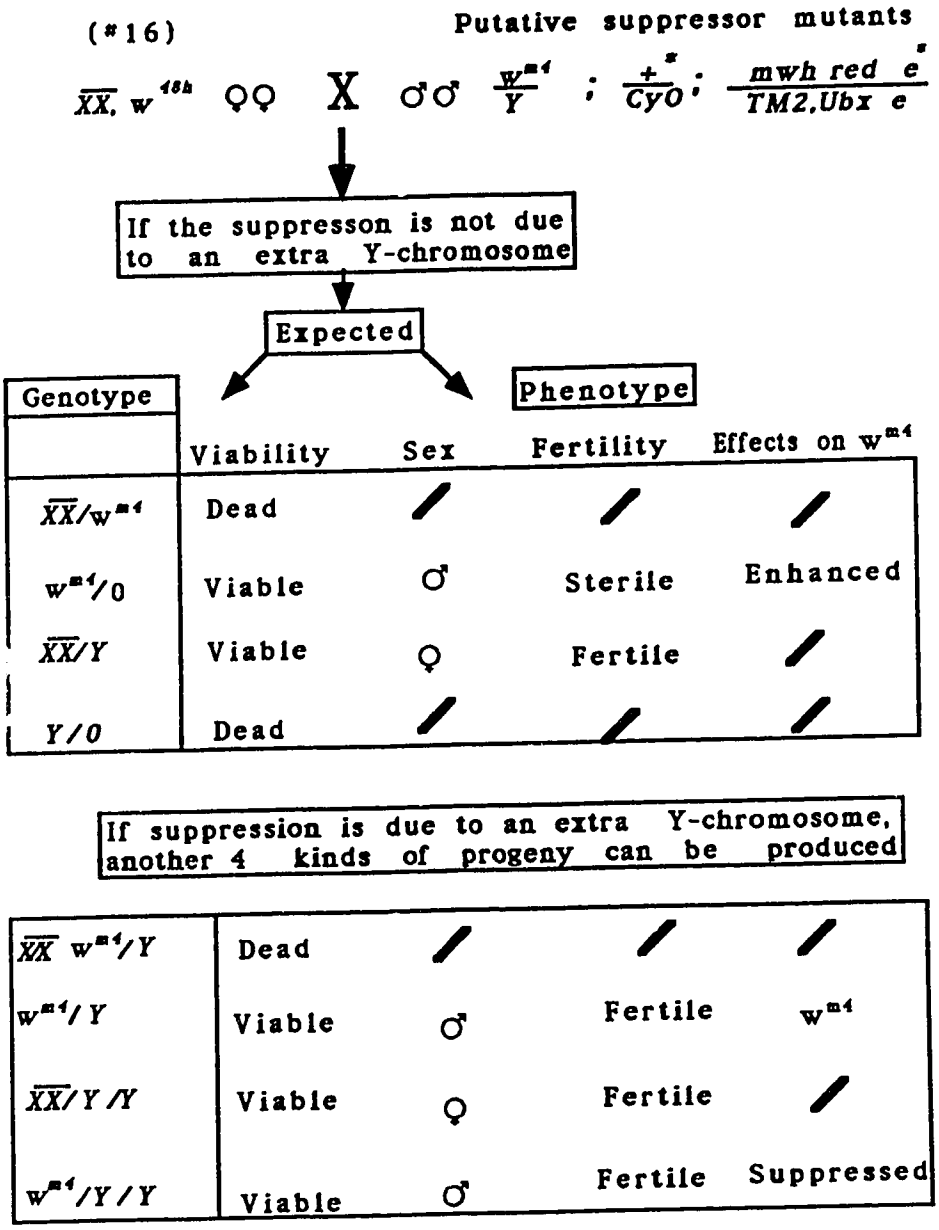


Figure 9. Cross to test for the presence of an extra Y-chromosome and assignment of suppressor mutations to the second or third chromosome. An explanation of this cross is seen in the Materials and Methods section 5. XX is the attached X chromosome. Asterisk (*) is the location of putative suppressor mutation supposed to be caused by the insertion of a pUChsneo P-element. w^{m4} represents $In(1)w^{m4}$, w^{m4}. Only male flies were used to confirm the location of suppressor mutations on the second or third chromosome.

This cross had another benefit. By counting the number of phenotypically different male flies from this cross and examining the segregation of Cy and Ubx from the suppressor phenotype, I could also determine whether the suppressor mutation was located on the second or third chromosome. If the suppressor mutations were located on the second chromosome, then all Cy^+ male progeny would be w^{m4} phenotype ($w^{m4}/0$; Su) and all Cy male progeny would be enhanced ($w^{m4}/0$). Similarly, if the suppressor was located on the third chromosome, then all Ubx^+ male progeny would be w^{m4} ($w^{m4}/0$; Su) and all Ubx male progeny would be enhanced ($w^{m4}/0$). If the suppression were due to an extra Y-chromosome, then both Cy and Cy^+ as well as Ubx and Ubx^+ progeny could be suppressed to a w^{m4} phenotype.

6. Estimation of Fly Number from Their Weight

Because of the large number of flies screened during my experiments, it was not practical for me to count every fly. Therefore, I collected all screened flies from each experiment into a pre-weighed beaker containing light mineral oil. This beaker was then reweighed with the screened flies. Based on the following experiments, the number of flies screened from their weights can be estimated.

At the beginning of my screening experiments, flies from one experiment were counted and different numbers of flies were put into four different beakers which contained light mineral oil. When the fly weights were plotted against the numbers of flies, I found a linear relationship. The results are shown in Figure 13.

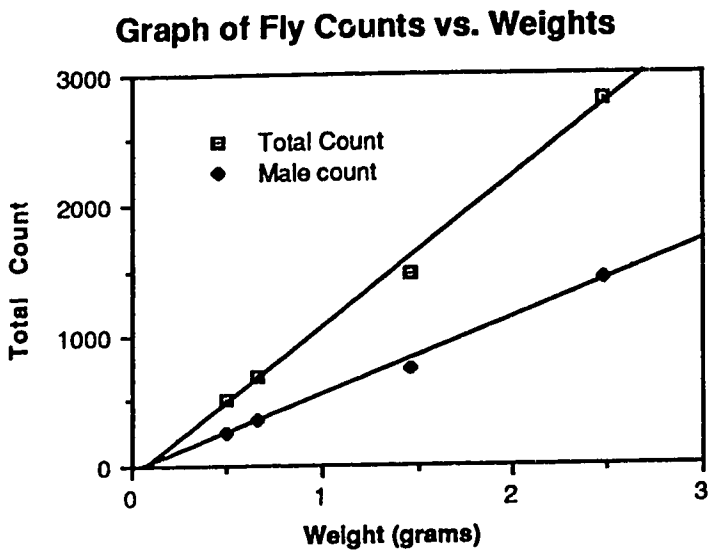


Figure 13: Graph of fly counts vs. weights showing the linear relationship between weight and numbers of flies.

Since the number of flies is linearly related to their weights, the average number of flies per gram was caculated. Number of Flies produced per gram = 5481/5.1 = 1075 flies/gram. The percentage of male flies = 2805/5481 = 51.12%. So the number of male flies screened per gram = 51.12% X 1075 = 549 male flies/gram.

7. Estimation of Eye Pigment

The amount of eye pigment in flies of various genotypes was measure. Crosses were made between males harboring a suppressor or enhancer mutation and virgin females with the genotype *In(1) w^{m4}, w^{m4}* (#67). The newly eclosed progeny were collected and kept in a vial with food for 5-6 days. Then the flies with different genotypes and sex were sorted , put into a 5 ml glass tube and stored in a -70°C freezer.

The eye pigment were measured according to the method of Ephrussi and Herold (1944). Frozen flies were vortexed vigorously for about 30 seconds to dissociate their body parts. Ten fly heads were picked out and put into a 1.5 ml Eppendorf tube. For each measurement, triplicate samples were prepared. Then 500 ul AMA (1% HCl in methyl alcohol) solution were then added to each Eppendorf tube. The tubes were shaken continuously on a rotatory shaker at room temperature. After 36-48 hours of shaking, 2.5 ul 1% H_2O_2 was added to each tube which was then incubated 90-120 minutes at room temperature. Then the absorbance of each sample at 470 nm was read. The data were subjected to statistical analysis.

8. Statistical Analyses

The PROC TTEST computer program from SAS/STAT software (Goodnight and Stanish, 1990) was used to statistically analyse the measurements of the effects of Su(var) and En(var) on the w^{m4} and B^{sv} variegation, as well as the effects of the Y-chromosome and parental source on w^{m4} eye pigment in male and female progeny.

PROC TTEST computes the t statistic based on the assumption that the variances of the two groups are equal, and computes an approximate t based on the assumption that the variances are unequal. For each t, the degrees of freedom and probability level are given. At the same time, an F' (folded) Statistic was computed to test for equality of the two variances. If the F' is > 5%, which indicates that the two variances are not significantly different, the t Statistic was used. But if the F' is < 5%, which indicates that the two variances are significantly different, the approximately t Statistic was used.

The computer caculated the two-tailed t-test, the P-values of one-tailed t-test was caculated by dividing the P-values from the two-tailed test by 2.

All of the caculations were performed by using University of Alberta computing systems-MTS (system EP190). To determine whether flies from one population, C1 (such as flies with genotype Su/+) contained significantly more eye pigment than flies from another population, C2 (such as Balancer/+ control flies), the null hypothesis H0: C1 = C2 and alternative hypothesis H1: C1 > C2 were proposed and tested. By calculating the t distribution with the degree of freedom (df.), the P-values were given by the computer. If the P-value < 0.05, which means that when H0: C1 = C2 is true, the probability that we have the observed data is less than 5%. Therefore, the H0 : C1 = C2 will be rejected according to conventional statistical levels of significance, and our alternative hypothesis H1 : C1 > C2 can be accepted.

9. Recombination Mapping

Suppressor or enhancer mutations were genetically mapped by standard mapping procedures. The dominant markers *Gl* (3-41.4), *Sb* (3-58.2) and *H* (3-69.5) were used to estimate the map positions of third chromosome suppressor mutations. The protocol crosses are given in Figure 10 (top panel). For mapping the second chromosome suppressor or enhancer mutations, the recessive markers *al* (2-0.01), *dp* (2-13.0), *b* (2-48.5), *c* (2-75.5), *px* (2-100.5) and *sp* (2-107.0) were used. The crossing scheme is shown in Figure 10 (bottom panel). In each mapping experiment, at least 1000 flies were scored.

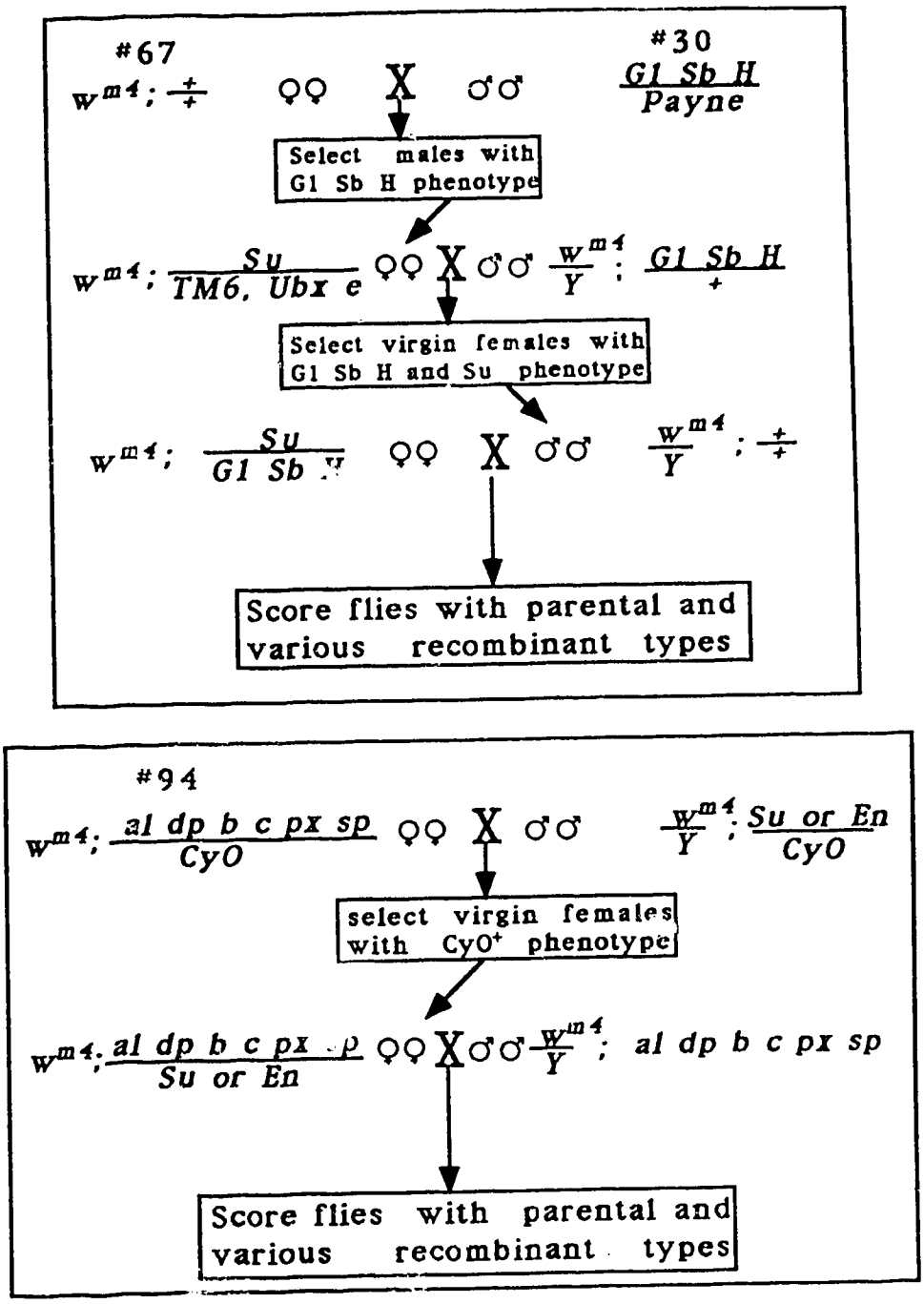


Figure 10. Cross scheme for mapping suppressor or enhancer mutations. Lab stock numbers are shown above the genotype when appropriate. w^{m4} represents $In(1)w^{m4}$, w^{m4}. The top panel showes the crosses for mapping the third chromosome suppressor mutations, and the bottom panel showes the crosses for the second chromosome suppressor or enhancer mutation mapping. The explanation of these crosses can be found in Materials and Methods.

10. Cytogenetic Analyses

The polytene chromosomes with suppressor or enhancer mutations were prepared from the salivary glands of F1 flies of crosses between wild-type (Oregon-R) virgin females and suppressor/balancer or enhancer/balancer males reared at room temperature. The salivary glands from late third-larval instar were squashed according to the method of Strickberger (1959). The polytene chromosomal banding patterns of heterozygous mutations were examined under Zeiss (West Germany) AXIOPHOT microscope for cytological aberrations. The revised polytene chromosome maps of Lefevre (1976) were used as a standard reference. For each mutation, at least 10 nuclei from at least 2 different slides (i.e. salivary glands from two different larvae) were carefully examined.

11. Genomic Southern Analyses

a). Isolation of Genomic DNA from Adult Flies

A modified Ish-Horowicz et al. (1979) method was used for isolating genomic DNA from adult flies. About 100 flies were homogenized in 1.5 ml solution I (10 mM Tris-Cl pH 7.5, 60 mM EDTA, 0.15 mM spermidine, 0.2 mg/ml pronase E (SigmaV), 50 ug/ml RNase A) and left at room temperature for 30 minutes. Then 1.5 ml solution II (0.2 M Tris-Cl pH 9, 30 mM EDTA, 2% SDS, 0.2 mg/ml pronase E) was added and the mixture was incubated at 37°C for 90 minutes. After incubation, the mixture was extracted twice with an equal volume of phenol, once with 1:1 phenol/chloroform and once with chloroform. About 200 µl 1.0 M NaCl was then added to the aqueous phase. The DNA was precipitated with 2.5 volumes of 95%

ethanol, and washed with 70% ethanol. After drying under vacuum, 100 µl TE was added.

b). Digestion of DNA with Restriction Enzymes

Approximately 5 µg genomic DNA of adult flies were digested with 10-20 units of restriction enzyme(s) in a 40 µl reaction mixture. Universal Restriction Buffer (URB) was used for both single and double digestions (Tartof and Hobbs, 1988) (33 mM Tris-acetate, pH 7.9, 66 mM Potassium acetate, 10 mM Magnesium acetate, 100 µg BSA/ml, 0.5 mM DTT and 4 mM Spermidine). The reaction mixture was incubated for 1-3 hours at 37°C, and then stopped by heating to 68°C for about 10 minutes. All restriction enzymes were from Bethesda Research Laboratories (BRL).

c). Electrophoresis

Digested genomic DNA was separated in a 0.8% agarose gel. Appropriate amounts of agarose were dissolved in TAE solution (40 mM Tris-acetate, 1 mM EDTA) in a microwave oven. After cooling to 65°C, ethidium bromide was added to a final concentration of 0.5-1 µg/ml before the gel was poured. The DNA samples were loaded into gel wells with gel loading buffer (0.1 M EDTA, 6 M urea, 2.5% sucrose and an appropriate amount of orange G dye). The *Hin*dIII digested λDNA fragments (23.13 kb, 9.42 kb, 6.68 kb, 4.36 kb, 2.32 kb, 2.03 kb, 0.564 kb and 0.125 kb) were used as molecular weight standards.

d). Southern Transfer

Genomic DNA, separated in an agarose gel, was transferred to Gene Screen Plus according to the supplier's instructions (NEN Research Products). After a photograph was taken under ultraviolet illumination, the gel was incubated in 0.4 N NaOH-0.6 M NaCl for about 30 minutes at room temperature with gentle agitation to denature the DNA. The gel was then neutralized by incubating it in a solution of 1.5 M NaCl-0.5 M Tris-HCl, pH 7.5 for 30 minutes. The DNA was transferred to a Gene Screen Plus membrane for 16-24 hours by the capillary procedure (Southern, 1975). After the transfer, the membrane was carefully removed from the gel and immersed in a 0.4 NaOH solution for 30-60 seconds to ensure complete denaturation of the immobilized DNA. After neutralization in a solution of 0.2 M Tris-HCl, pH 7.5-2 x SSC, the membrane was allowed to dry at room temperature, and then kept in a plastic bag until used.

e). Mini-preparation of Plasmid DNA

Mini-preparation of plasmid DNA was carried out according to the procedures of Tartof and Hobbs (1988). About 1.5 ml of an overnight bacterial cell culture in T-broth containing 100 µg/µl ampicillin was transferred to a 1.5 ml microfuge tube and spun 30 seconds at 14,000 rpm. The supernatant was discarded and the pellet was resuspended in 90 µl lysis buffer (50 mM glucose, 10 mM EDTA, 25 mM Tris-HCl pH 8.0). Then 10 µl of 20 mg/ml freshly prepared lysozyme (dissolved in lysis buffer) was then added to the cell suspension, and then it was placed on ice. After 30 minutes, 200

µl of an alkaline SDS solution (0.2 M NaOH, 1% SDS) was added and incubated on ice for another 5 minutes. At that time, the solution becomes viscous. 150 µl of 3 M KAc was added, and put in ice for 10 minutes and then centrifuged for 5 minutes. The supernatant was transferred to a new microfuge tube and extracted once with an equal volume phenol, then phenol-chloroform (1:1) and chloroform. The DNA was precipitated by adding 1 ml 95% ethanol, washed with 70% ethanol and dried for about 30 minutes. The DNA was resuspended in 50 µl TE buffer.

f). Isolation of DNA fragments from low-melting-point (LMP) gels

About 2 µg plasmid pπ25.1 (Spradling and Rubin, 1982) DNA was digested with *Bam*HI and *Xho*I in a 20 µl reaction mixture, and then separated on a 1% LMP agarose gel. When sufficient resolution was achieved, two smaller DNA fragments were cut out of the gel with a razor blade and then stored in an Eppendorf tube. An appropriate amount (1.5 ml/gm gel) of double distilled water was added to the gel piece. The tube was boiled for 10 minutes, and then kept at - 20°C. The isolated DNA fragments were used to probe the genomic Southern blots of suppressor and enhancer mutations.

g). Random Primed DNA Labelling

The probes for genomic Southern blots were labelled by using the Random Primed DNA Labelling Kit (Boehringer Mannheim). 50-200 ng *Mbo*I-digested pUC19N plasmid DNA or isolated P-element fragments was added to a 20 µl reaction mixture containing 1 µl dATP (0.5 mmol/l), 1 µl dGTP (0.5 mmol/l), 1 µl dTTP (0.5 mmol/l),

2 µl hexanucleotide mixture in 10 X concentrated reaction buffer, 5 µl [α^{32}P] dCTP (3000 Ci/mmol), and 1 µl Klenow enzyme and incubated at 37°C for 2 hours to overnight. The reaction was stopped by adding 2 µl EDTA, 0.2 mol/l (pH 8.0). The unincorporated nucleotides were removed by passing through Sephadex G-50 column, as described in Maniatis et al. (1982).

h). Southern Hybridizations

The Gene Screen Plus membranes (15 x 20 cm) were prehybridized at 65°C for at least 2 hours in a plastic bag with 50 ml of hybridization solution with constant and gentle shaking. The hybridization solution contained 15 ml of 20 x SSC (3 M Sodium Chloride, 0.3 M Sodium Citrate), 2.5 ml of 1 M Na_3PO_4, pH 7.0 and 5 ml of 10% SDS. The appropriate amount of ^{32}P-labelled probe was added and the plastic bag was resealed. The hybridization reaction continued for 12-24 hours at 65°C with constant shaking.

After hybridization, the membrane was washed twice with 2 X SSC, 0.1% SDS at room temperature, twice with 2 X SSC, 0.1% SDS at 65°C for 30 minutes each, twice with 0.1 X SSC, 0.1% SDS at 65°C for 30 minutes each, and then the damp membrane was placed in into a plastic bag for autoradiographly.

To strip the probe from the immobilized genomic DNA on the membrane, so that another probe could be used, the Gene Screen Plus membrane was incubated in 200 ml of 0.4 N NaOH at 42°C for 30 minutes with gentle agitation and then incubated in 200 ml of 0.1 X SSC, 0.1% SDS, 0.2 M Tris-HCl, pH 7.5 at 42°C for 30 minutes. The

rehybridization with the second probe was carried out as described above.

i). Autoradiography

The membrane (in a plastic bag) was exposed to a Kodak X-OMAT AR film, in an X-Ray cassette containing an intensifying screen, at -70°C . For the genomic DNA southern blotting, at least 1 day exposure was required. After exposure, the film was developed using Kodak X-Ray film GBX developer (3 minutes), stop solution (30 seconds) and GBX fixer (2 minutes). The film was washed in a running water for about 5 minutes and then dried at room temperature.

III. Results

In this chapter, I am going to describe the isolation of 66 dominant suppressor mutations, which represent at least 30 independently isolated mutations, and four enhancer mutations. Then I am going to describe the characterization of 20 of these suppressor and 4 enhancer mutations. During the study, some mutations were lost, only 20 independently isolated suppressor mutations are now available. They are Su(var)KD201, 203, 206, 208, 209, 211, 306, 310, 322, 328, 334, 335, 336, 337, 338, 340, 341, 343, 345, 354, and En(var)KD201, 202, 203, 204. The characterization includes:

1). Viability and fertility of Suppressor and enhancer mutations as homozygotes.

2). Complementation analysis among suppressor mutations and enhancer mutations.

3). Effects of suppressor and enhancer mutations on two different variegating alleles w^{m4} and B^{sv}.

4). Cytogenetical analyses of suppressor and enhancer mutations using polytene chromosomes.

5). Recombination mapping of Su(var)KD306, 328, 340 and En(var)KD201.

6). Phenotypic interaction between suppressor and enhancer mutations.

7). Genomic Southern analyses using pUC19N and P-element sequences as probes.

Finally, two additional experiments are presented:

1). Effects of the parental source on w^{m4} variegation.

2). Effects of different Y-chromosomes on w^{m4} variegation.

1. Screening for Suppressor Mutations of w^{m4}

To isolate dominant autosomal suppressor mutations of w^{m4} variegation by pUChsneo P-element mutagenesis, a two generation cross-scheme was used as described in Materials and Methods section 3. By mating virgin females, having the pUChsneo P-element on their X-chromosome, to males bearing a stable transposase-producing Δ2-3 P-element, the first generation potential dysgenic males were obtained. They were crossed to w^{m4} virgin females to produce progeny which were screened for suppressor mutations. The results of screening for suppressor mutations of the variegating allele w^{m4} are presented in Table 2.

In the beginning of screening for suppressor mutations, cross scheme A and cross scheme B were used (see Figure 3 and 4). A total of 47 putative suppressor mutants were found from one tray for each cross scheme. Since the further analysis was difficult (for reasons, see Materials and Methods section 3), and in these experiments the next generation crosses were severely contaminated by bacteria, no suppressor mutations were recovered from these experiments.

Because of the problem encountered in the cross scheme A and B, cross scheme C was undertaken (see Figure 5). Sixteen putative suppressor mutations were recovered from an estimated 11,400 males. Putative mutations were individually crossed to $In(1)w^{m4}$;

Notes of Table 2

a). Each experiment contains one tray with 16 bottles.

b). Cross schemes used in the screening for suppressor mutations of PEV are shown in the Figure3, 4, 5, and 6.

c). Non-transmitting putative suppressor mutations.

d). Transmitting putative suppressor mutations.

e). Number of suppressor mutation stocks established.

f). Number of independently-isolated mutations from different bottles.

g). From these two experiments, 47 putative suppressor mutation males were obtained. Subsequent crosses were infected with bacteria. A question mark indicates that the number is unknown.

h). In these experiments, I found bottles with many mutants. For example, Expt. # 16 had one bottle with 8 putative mutants and Expt. # 24, 28, 29 have bottles with more than 10 putative mutants. All of putative mutants from each bottle have the same phenotype and were located on the same chromosome. Each set was probably the same premeiotic mutation. Initially only 8 stocks from each experiments were established. But now only two stocks for each cluster of mutations were maintained.

i). Sub-total of cross scheme B and Sub-total of cross scheme D.

Table 2 Screening for Suppressor Mutations of PEV

Expt. #[a]	Cross Scheme used[b]	Weight of flies scored g	# of males scored	Number of male putative mutants					# of[e] stocks	# of[f] mutant
				Total	Sterile	Extra Y	Non-T[c].	Transmit[d]		
1g	A	5.1	2802	20	?	?	?	?	0	0
2g	C	8.2	4516	27	?	?	?	?	0	0
7	B	2.33	1280	4	2	0	2	0	0	0
12	B	8.49	4669	5	1	1	3	0	0	0
13	B	9.88	5428	7	4	3	0	0	0	0
SubT[i]		20.70	11377	16	7	4	5	0	0	0
3	D	3.11	1709	1	0	0	1	0	0	0
4	D	6.22	3418	8	2	0	4	2	2	1
5	D	2.1	1154	9	3	1	5	0	0	0
6	D	2.1	1154	5	2	0	3	0	0	0
8	D	9.18	5044	6	4	0	2	0	0	0
9	D	5.1	2802	7	2	0	5	0	0	0
10	D	4.24	2330	2	1	0	1	0	0	0
11	D	7.16	3934	14	6	1	5	2	2	1
14	D	11.54	6340	15	8	0	7	0	0	0
15	D	15.49	8510	20	10	0	10	0	0	0
16	D	16.97	9324	22	9	0	5	8[h]	8	1
17	D	19.42	10675	31	15	1	15	2	2	2
18	D	15.91	8741	18	4	2	7	5	5	3
19	D	13.83	7598	22	8	1	11	2	2	2
20	D	10.72	5890	21	5	1	14	1	1	1
21	D	12.49	6862	14	4	1	8	1	1	1
22	D	10.36	5692	13	7	1	5	0	0	0
23	D	15.56	8549	18	1o	0	8	0	0	0
24	D	15.84	8702	38	16	1	7	14[h]	9	2
25	D	15.16	8329	15	4	2	9	0	0	0
26	D	17.16	9428	20	7	3	10	0	0	0
27	D	22.8	12526	34	13	1	17	3	3	3
28	D	12.52	6879	40	15	1	2	22[h]	9	2
29	D	14.87	8170	36	11	2	7	16[h]	10	3
30	D	9.68	5318	15	8	1	6	0	0	0
31	D	14.68	8065	17	12	0	3	2	2	1
32	D	17.09	9389	19	8	0	11	0	0	0
33	D	14.23	7818	9	5	0	3	1	1	1
34	D	12.93	7104	16	11	1	2	2	2	2
35	D	12.9	7087	13	10	1	2	0	0	0
36	D	10.78	5923	14	9	0	5	0	0	0
37	D	9.16	5033	8	8	0	0	0	0	0
38	D	9.46	5197	15	11	1	3	0	0	0
39	D	8.27	4544	4	4	0	0	0	0	0
40	D	5.73	3148	10	3	2	3	2	2	1
41	D	5.83	3203	7	5	0	2	0	0	0
42	D	6.52	3582	8	5	1	1	1	1	1
43	D	5.86	3219	10	4	1	0	4	4	2
44	D	4.92	2703	7	5	2	0	0	0	0
45	D	5.86	3219	7	7	0	0	0	0	0
SubT[i]		433.76	238,308	608	281	27	210	90	66	30
Total		467.76	256,987	671	288	31	210	90	66	30

CyO/Xa/TM2, Ubx e (#58) virgin females. Of the 16 putative mutations, 7 were not fertile, 4 were due to an extra Y-chromosome and 5 were not transmitted. Thus no transmitting suppressor mutations were recovered from cross scheme C. This could be due to a low amount of transposase activity in Δ2-3 P-element of *w; Sb e Δ2-3(99B)/TM6* (#27).

For the reasons outlined in Materials and Methods section 3, cross scheme D was used and 40 experiments were set up (see figure 6). In total, 238,308 potential suppressor bearing males were screened and from them 608 putative suppressor mutations were obtained. By crossing them individually to *In(1)w^{m4}; CyO/Xa/TM2, Ubx e* (#58) virgin females and to *XX, w^{48h}* (#16), I found that 281 (46%) were sterile, 27 (4%) were probably due to an extra Y-chromosome, 90 (15%) were transmitting suppressor mutations and 210 (35%) were not transmitting, and probably due to the variability in variegated expression of white gene in *w^{m4}*.

2. Established Suppressor Mutation Stocks

From the 90 transmitting suppressor mutations, 66 lines of suppressor stocks were established. The other 24 transmitting suppressor mutations were discarded because they were probably duplicate premeiotic events as explained in Table 2. Among these 66 suppressor stocks, those established from suppressor mutations isolated from the same bottle were called clusters. These include the second chromosome clusters II_1, II_2, II_5, II_6, and the third chromosome clusters III_1, III_2, III_3, III_4, III_6, III_{20}. The remaining stocks were from single, independent mutations, each in their own

Table 3 Stocks of Suppressor Mutations Established

Allele Names Su(var)KD	From # of Experiments[a] Expt.#	Bottle#	Independent (single) or Group (clusters)[b]	Balanced by	Chromosome assignment[c]	Fate[d]
201	4	8	II_1	CyO	II	Retained
202	4	8	II_1	CyO	II	Lost
203	11	9	II_2	CyO	II	Retained
204	11	9	II_2	CyO	II	Lost
205	17	5	II_3	CyO	II	Lost
206	34	15	II_4	CyO	II	Retained
207	31	14	II_5	CyO	II	Lost
208	31	14	II_5	CyO	II	Retained
209	40	3	II_6	CyO	II	Retained
210	40	3	II_6	CyO	II	Lost
211	42	16	II_7	CyO	II	Retained
301	16	12	III_1	TM2 and TM6	III	Discard
302	16	12	III_1	TM2 and TM6	III	Discard
303	16	12	III_1	TM2 and TM6	III	Retained
304	16	12	III_1	TM2 and TM6	III	Discard
305	16	12	III_1	TM2 and TM6	III	Discard
306	16	12	III_1	TM2 and TM6	III	Retained
307	16	12	III_1	TM2 and TM6	III	Discard
308	16	12	III_1	TM2 and TM6	III	Discard
309	24	11	III_2	TM2 and TM6	III	Discard
310	24	11	III_2	TM2 and TM6	III	Retained
311	24	11	III_2	TM2 and TM6	III	Discard
312	24	11	III_2	TM2 and TM6	III	Discard
313	24	11	III_2	TM2 and TM6	III	Retained
314	24	11	III_2	TM2 and TM6	III	Discard
315	24	11	III_2	TM2 and TM6	III	Discard
316	24	11	III_2	TM2 and TM6	III	Discard
317	28	11	III_3	TM2 and TM6	III	Discard
318	28	11	III_3	TM2 and TM6	III	Discard
319	28	11	III_3	TM2 and TM6	III	Discard
320	28	11	III_3	TM2 and TM6	III	Retained
321	28	11	III_3	TM2 and TM6	III	Discard
322	28	11	III_3	TM2 and TM6	III	Retained
323	28	11	III_3	TM2 and TM6	III	Discard
324	28	11	III_3	TM2 and TM6	III	Discard
325	29	10	III_4	TM2 and TM6	III	Discard

326	29	10	III_4	TM2 and TM6	III	Retained
327	29	10	III_4	TM2 and TM6	III	Discard
328	29	10	III_4	TM2 and TM6	III	Retained
329	29	10	III_4	TM2 and TM6	III	Discard
330	29	10	III_4	TM2 and TM6	III	Discard
331	29	10	III_4	TM2 and TM6	III	Discard
332	29	10	III_4	TM2 and TM6	III	Discard
333	24	13	III_5	TM2	III	Lost
334	17	6	III_6	TM2	III	Retained
335	18	6	III_7	TM2	III	Retained
336	18	11	III_8	TM2	III	Retained
337	19	4	III_9	TM2	III	Retained
338	19	6	III_{10}	TM2	III	Retained
339	27	2	III_{11}	TM2	III	Lost
340	27	6	III_{12}	TM2	III	Retained
341	34	15	III_{13}	TM2	III	Retained
342	21	14	III_{14}	TM2	III	Lost
343	20	7	III_{15}	TM2	III	Retained
344	18	7	III_{16}	TM3	III	Lost
345	18	7	III_{16}	TM3	III	Retained
346	18	7	III_{16}	TM3	III	Retained
347	29	7	III_{17}	TM3	III	Lost
348	29	4	III_{18}	TM3	III	Lost
349	28	13	III_{19}	TM3	III	Lost
350	43	3	III_{20}	TM3	III	Lost
351	43	3	III_{20}	TM3	III	Lost
352	43	3	III_{20}	TM3	III	Lost
353	43	13	III_{21}	TM3	III	Lost
354	33	7	III_{22}	TM2	III	Retained
355	27	3	III_{23}	TM2	III	Lost

Notes

a). One experiment is composed of on tray crosses with 16 bottles.

b). Clusters arise when more than one mutations are isolated from the same bottle. Single mutations were isolated from different bottles.

d). II is the second chromosome and III is the third chromosome.

e). Retained means that the stocks are retained in the lab. Whereas lost means that the stocks were lost. Discarded means that the stocks were discarded for the reasons mentioned in the Materials and Methods section 4.

bottle. In total there were at least 30 suppressor mutations independently isolated from different bottles. The frequency of independent mutations recovered is 30/238,000 =1.26 x 10^{-4}.

From each cluster, only 2 lines were kept since the suppressor mutations within the same cluster had indistinguishable phenotypes and were assigned to the same chromosome, they were all likely to be the same mutation. Also, while maintaining these stocks, some mutation stocks grew very poorly, especially those stocks having suppressor mutations balanced by TM3 (see Methods and Materials 4), so some stocks were lost.

I now have retained 32 suppressor mutation stocks representing at least 20 independent suppressor mutations. Among them, 6 suppressor mutations were located on the second chromosome and 14 were located on the third chromosome (see Table II notes). All the stocks I established, and the remaining stocks are listed in Table 3.

3. En(var) Mutations Derived from Su(var) Stocks.

Initially, the stocks for the second chromosome suppressor mutations were homozygous for the third chromosome *mwh red e*. This chromosome carries a *red* gene mutation, which can affect the eye color and therefore affect the phenotype of w^{m4} variegation. To replace this third chromosome, males from these stocks were crossed to w^{m4}; *CyO/Xa* (#62) virgin females. However in subsequent generations, the suppressor mutations became unstable, in that revertants were spontaneously generated.

Additionally, I recovered several male flies with completely white eyes. These flies were crossed to w^{m4}; *CyO/Xa/TM2,Ubx e* (#58) to make a balancer stock for the putative enhancer mutations, just as in Figure 8 except that * now indicates a putative enhancer mutation. These enhancer mutation stocks are shown in Table 4. By this cross (see Figure 8), all the enhancer mutations were assigned to the second chromosome since they segregated from CyO; just like the suppressors from which they were derived.

Table 4

Enhancer mutations derived from Suppressor mutation stocks

En(var) mutations	Parental Suppressor mutations Stock	Group	Segregated from and Balanced by	Chromosome Assignment
En(var)KD201	Su(var)KD201	II_1	CyO	II
En(var)KD202	Su(var)KD202	II_1	CyO	II
En(var)KD203	Su(var)KD203	II_2	CyO	II
En(var)KD204	Su(var)KD209	II_6	CyO	II

4. Viability and Fertility of Su(var) and En(var) Mutations as Homozygotes

Since most modifier mutations of PEV are recessive lethals, and those which are recessive viable are often recessive sterile (Sinclair *et al.*, 1983; Locke *et al.*, 1988), suppressor and enhancer homozygotes were tested for viability and fertility.

To determine the viability of the suppressor and enhancer mutations as homozygotes, Su(var)/balancer or En(var)/balancer flies were sib-crossed, and for each cross at least 300 progeny were examined. If no homozygous flies were found, the viability should be less than 1%. If some homozygous mutation flies were found, the

Table 5 Viability and Fertility of Suppressor and Enhancer Mutations as Homozygotes

Mutations[a]	Viability of homozygotes			Fertility of homozygotes	
Su(var)KD	Number[b]	Percent[c]	Description	Males	Females
201	96/474	41%	semi-lethal	die as larvae	sterile (no eggs layed)
203	53/[illegible]	23%	semi-lethal	die as larvae	sterile (no eggs layed)
208	34/228	30%	semi-lethal	fertile	sterile (no eggs layed)
209	23/325	14%	semi-lethal	fertile	sterile (no eggs layed)
211	31/337	18%	semi-lethal	fertile	sterile (no eggs layed)
206	0/502	0	lethal	ND[d]	ND
306	0/678	0	lethal	ND	ND
310	0/512	0	lethal	ND	ND
322	0/410	0	lethal	ND	ND
328	0/598	0	lethal	ND	ND
340	0/805	0	lethal	ND	ND
334	0/478	0	lethal	ND	ND
341	0/713	0	lethal	ND	ND
343	0/328	0	lethal	ND	ND
345	0/415	0	lethal	ND	ND
335	98/215	91%	viable	fertile	die as larvae
336	180/412	87%	viable	fertile	die as larvae
337	153/312	85%	viable	sterile	die as larvae
338	231/512	90%	viable	fertile	die as larvae
354	8/193	8%	semi-lethal	sterile	die as larvae
En(var)KD					
201	0/549	0	lethal	ND	ND
202	0/412	0	lethal	ND	ND
203	0/618	0	lethal	ND	ND
204	0/385	0	lethal	ND	ND

Notes:

a. At the time of this test, only 20 independently-isolated mutations were available.

b. Number A/B: A is the flies with homozygous mutations and B is the number of flies with Su(var)/Balancer or En(var)/Balancer genotype.

c. The percent of expected viable homozygous flies.

d. ND represent Not Done because they are lethal as homozygotes.

homozygote viability can be calculated according to the following formula:

Viability = Observed homozygotes / Expected homozygotes
[Expected homozygotes = 1/2 Su(var)/balancer or En(var)/balancer flies examined].

The second chromosome Su(var) and En(var) mutations were balanced by the CyO chromosome, which has a dominant marker, Cy. If the homozygotes of Su(var) and En(var) mutations are viable, Cy^+ flies will be generated in the sib-crosses. The third chromosome balancers TM2 and TM6 have a dominant marker, Ubx. The appearance of Ubx^+ flies in a third chromosome Su(var)/balancer sib-cross would indicate that these mutations are viable as homozygotes.

For determining the fertility of the homozygous viable mutations, the male and female homozygotes were collected and then crossed to wild type (Oregon-R) virgin females and males respectively in about 5 vials for each cross. After about 10 days, if no progeny are produced, they were considered sterile as homozygotes.

The results are shown in Table 5. Of the 6 independently-isolated second chromosome suppressor mutations, only one, Su(var)KD206, is a recessive lethal. The viability of the other 5 suppressor mutations, Su(var)KD201, 203, 208, 209 and 211, as homozygotes ranges from 14% to 41%. When homozygous females of these 5 suppressor mutations were crossed to wild-type (Oregon-R) males, no eggs were produced. This indicated that all 5 suppressor mutations are sterile as homozygous females. However, when homozygous males were crossed to wild-type (Oregon-R) virgin

females, Three mutations, Su(var)KD208, 209, 211, appeared fully fertile. From the crosses involving Su(var)KD201 or 203 males and wild-type virgin females, some larvae could be found in the vials, but they died before reaching the pupal stage. This observation, Su(var)/Su(var) males giving rise to progeny that die as larvae, is unusual. Therefore, these crosses were repeated several times, with the same results each time. It implies a dominant paternal effect which is difficult to explain. To date I still do not understand the reason behind these observations.

When the viability of the third chromosome homozygous suppressor mutations was examined, I found that 9 (Su(var)KD306, 310, 322, 328, 334, 340, 341, 343 and 345) were recessive lethals and only 5 (Su(var)KD335, 336, 337, 338 and 354) were viable as homozygotes. In contrast to the second chromosome suppressor mutations, which were mainly recessive semi-lethals, these third chromosome mutations were fully viable as homozygotes. The Su(var)KD335, 336, 338 males were fertile as homozygotes, while the homozygous males of Su(var)KD337 and 354 were sterile. When homozygous females of these mutations were crossed to wild type (Oregon-R) males. Several days elapsed, some larvae could be found, which did not develop further. It seems that these Su(var) mutation-bearing chromosomes carry a recessive maternal effect mutation, which can affect the development of the progeny. Therefore the zygotes produced by these homozygous females and wild type males could develop only as far as the larval stage and not to the pupal or adult stages.

Determination of the viability and fertility of the homozygous enhancer mutations derived from suppressor mutation stocks (see Results section 4), showed that they were all recessive lethal (see Table 5). This supports the idea that an additional mutation has occurred because the suppressor mutations, from which they were derived, were all homozygous viable.

The above experiments indicate that most of the Su(var) and En(var) mutations are lethal or sterile as homozygotes. It was important to know whether these lethal or sterile effects were associated with the Su(var) and En(var) mutations or were due to the second site mutations. Since the above experiments only tested the viability and fertility of the whole chromosome and not the mutations specifically, more experiments had to be done to show a possible association between the lethality or sterility and the Su(var) or En(var) mutations. Within limits, this can be done by testing appropriate recombinant chromosomes to determine whether the Su(var) or En(var) mutations and the lethality and sterility phenotypes were linked. This analysis was done with Su(var)KD340.

During the recombination mapping of Su(var)KD340, four different recombinant males with Su(var) or without Su(var) mutations were selected from the progeny of a cross between w^{m4}; + *Su* + +/*Gl* Su^{+} *Sb H* females and males w^{m4}; +/+ (#67). These four recombinants are w^{m4}/*Y*; + Su^{+} *Sb H*/+ *Su* + + and w^{m4}/*Y*; *Gl Su*+ *Sb* +/+ Su^{+} + + without suppressor mutation; w^{m4}/*Y*; + *Su* + *H*/+ Su^{+} + + and w^{m4}/*Y*; *Gl Su* + +/+ Su^{+} + + with suppressor mutation. Then these males were crossed to Su(var)KD340/TM2 virgin females to

Su(var)KD340 × Recombinants

$w^{m4}; \frac{Su}{TM6,\ Ubx\ e}$ ♀♀ × ♂♂ $\frac{w^{m4}}{Y}$; $\frac{+\ Su\ +\ H}{+\ Su^{+}\ +\ +}$

Score flies with different genotype and Phenotype

Genotype	Phenotype	Number
$w^{m4}; \frac{Su}{+\ Su\ +\ H}$	Su H	0
$w^{m4}; \frac{Su}{+\ Su^{+}\ +\ +}$	Su	20
$w^{m4}; \frac{+\ Su\ +\ H}{TM6,\ Ubx\ e}$	Su H Ubx	16
$w^{m4}; \frac{+\ Su^{+}\ +\ +}{TM6,\ Ubx\ e}$	Ubx	29

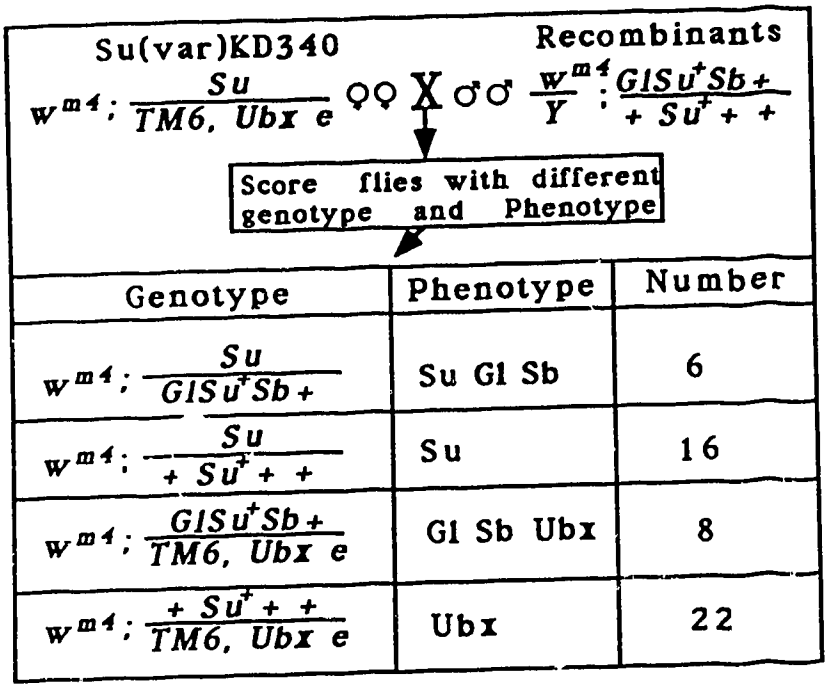
Su(var)KD340 × Recombinants

$w^{m4}; \frac{Su}{TM6,\ Ubx\ e}$ ♀♀ × ♂♂ $\frac{w^{m4}}{Y}$; $\frac{Gl\ Su\ +\ +}{+\ Su^{+}\ +\ +}$

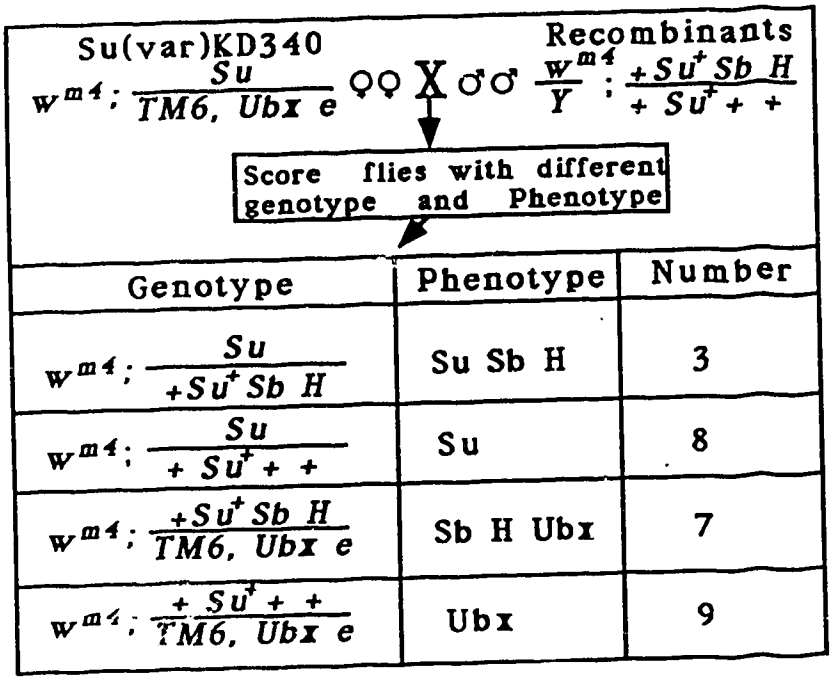
Score flies with different genotype and Phenotype

Genotype	Phenotype	Number
$w^{m4}; \frac{Su}{Gl\ Su\ +\ +}$	Su Gl	0
$w^{m4}; \frac{Su}{+\ Su^{+}\ +\ +}$	Su	46
$w^{m4}; \frac{Gl\ Su\ +\ +}{TM6,\ Ubx\ e}$	Gl Su Ubx	17
$w^{m4}; \frac{+\ Su^{+}\ +\ +}{TM6,\ Ubx\ e}$	Ubx	47

Figure 11. Crosses and results showing that the recessive lethality of Su(var)KD340 was associated with the suppressor mutation. The recombinants $w^{m4}/Y;\ +\ Su\ +\ H/+\ Su^{+}\ +\ +$ and $w^{m4}/Y;\ Gl\ Sb\ +\ +/+\ Su^{+}\ +\ +$ with the suppressor mutations on the chormosome were selected from cross between $w^{m4};\ Gl\ Sb\ H\ Su^{+}/+\ +\ +\ Su$ females and $w^{m4};\ +/+$ (#67) males. w^{m4} represents $In(1)\ w^{m4},\ w^{m4}$. The explanation of these crosses and results can be found in Results.



Genotype	Phenotype	Number
$w^{m4}; \frac{Su}{Gl Su^{+} Sb +}$	Su Gl Sb	6
$w^{m4}; \frac{Su}{+ Su^{+} + +}$	Su	16
$w^{m4}; \frac{Gl Su^{+} Sb +}{TM6, Ubx\ e}$	Gl Sb Ubx	8
$w^{m4}; \frac{+ Su^{+} + +}{TM6, Ubx\ e}$	Ubx	22



Genotype	Phenotype	Number
$w^{m4}; \frac{Su}{+ Su^{+} Sb\ H}$	Su Sb H	3
$w^{m4}; \frac{Su}{+ Su^{+} + +}$	Su	8
$w^{m4}; \frac{+ Su^{+} Sb\ H}{TM6, Ubx\ e}$	Sb H Ubx	7
$w^{m4}; \frac{+ Su^{+} + +}{TM6, Ubx\ e}$	Ubx	9

Figure 12. Crosses and results showing that the recessive lethality of Su(var)KD340 was associated with the suppressor mutation. The recombinants w^{m4}/Y; *Gl* Su^{+} *Sb* +/+ Su^{+} + + and w^{m4}/Y; + Su^{+} *Sb H*/+ Su^{+} + + without the suppressor mutations on the chormosome were selected from cross between w^{m4}; *Gl Sb H* Su^{+}/+ + + *Su* females and w^{m4}; +/+ (#67) males. w^{m4} represents *In(1)* w^{m4}, w^{m4}. The explanation of these crosses and results can be found in Results.

determine whether the lethality is always linked with Su(var)KD340. The crosses and results are shown in Figures 11 and 12.

When the recombinant males, with a suppressor mutation present on one chromosome, were crossed to w^{m4}; *Su(var)KD340/TM6, Ubx e* only three kinds of flies were found (see Figure 12). The w^{m4}; + *Su* + *H*/+ *Su* + + and w^{m4}; *Gl Su* + +/+ *Su* + + flies were lethal. On the other hand, the crosses between recombinant males without a suppressor mutation on their chromosomes and w^{m4}; *Su(var)/TM6, Ubx e* produce four kinds of flies (see Figure 11). The above results indicate that on the Su(var)KD340 chromosome the recessive lethality of Su(var)KD340 was linked to the suppressor mutation.

5. Complementation Analyses of Su(var) Mutations

Complementation tests between the dominant suppressor mutations can not be done. Consequently, the determination of lethal complementation groups would only be possible if we assume that the recessive lethality and dominant suppressor mutations are caused by the same mutations, since at this time it is still not clear whether the recessive lethality is caused by the mutation or not (see Results 4 for an explanation).

Of the 6 second chromosome Su(var) mutations, only Su(var)KD206 was lethal as a homozygote. Thus no complementation tests could be done with the second chromosome Su(var) mutations.

Of the 14 third chromosome Su(var) mutations, there are 9 mutations (Su(var)KD306, 310, 322, 328, 340, 334, 341, 343 and 345), which are lethal as homozygotes. Are any of these 9 Su(var)

mutations allelic? To answer this question, crosses were made to test for complementation of the resessive lethal phenotype among these mutations. In all crosses, at least 300 progeny were examined for the viability of flies bearing both Su(var) mutations.

All of the recessive-lethal mutations were maintained over a third chromosome balancer with the dominant marker Ubx. When the crosses were made between these mutation stocks, the appearance of Ubx^+ progeny indicated that the trans-heterozygote was viable and that the two lethal chromosomes can complement each other. Thus, they belong to different lethal complementation groups.

The cross results show that the 9 mutations probably belong to 4 different lethal complementation groups (Table 6). Crosses made among Su(var)KD306, 310, 322, 328 and 340 produced no Ubx^+ flies in several hundred progeny, indicating these mutations belong to the same lethal complementation group designated group I.

Next, virgin females of Su(var)KD328, A representative of the lethal complementation group I, were crossed to males of Su(var)KD334, 341, 343 and 345, respectively. Su(var)KD334, 341, 343 and 345 can complement the lethality of Su(var)KD328. Finally crosses among these mutations were made, and another three complementation groups were identified. In total the 9 recessive lethal mutations define 4 lethal complementation groups.

Table 6

Complementation analyses the third chromosome homozygous lethal suppressor mutations

Su(var)KD	GroupI					GroupII	GroupIII	Group IV	
Cross	306[a]	310	322	328	340	334	341	343	345
306	0/358[b]	0/815	0/613	0/512	0/412	-	-	-	-
310		0/664	0/549	0/697	0/404	-	-	-	-
322			0/572	0/738	0/497	-	-	-	-
328				0/522	0/550	63/170	85/173	61/162	93/192
340					0/472	-	-	-	-
334						0/512	26/163	35/161	30/156
341							0/718	50/181	39/172
343								0/485	0/330
345									0/318

Notes:

a. 306, 310 etc. represent Su(var)KD306 and Su(var)KD310 etc.

b. A/B: A is the Ubx+ progeny with genotype Su(var)1/Su(var)2 and B Ubx progeny with genotype Su(var)1/Balancer Ubx or Su(var)2/Balancer Ubx.

6. Complementation Analyses of En(var) Mutations

All four enhancer mutations, unlike the suppressor mutations from which they were derived, are recessive lethal. To test for complementation of their recessive lethality, crosses were made among these four En(var) mutations. All four En(var) mutations were balanced by a CyO balancer with a dominant Cy marker. The appearance of Cy^+ progeny from a cross would indicate that two mutation-bearing chromosomes can complement each other's lethality while no Cy^+ progeny would indicate no complementation for the recessive lethality.

The lethal complementation cross results are shown in Table 7. Among more than 300 progeny scored, no Cy+ flies were found in every cross. This result indicates that these four enhancer mutations can not complement each other's lethality. Therefore, They all belong to the same lethal complementation group.

Table 7

Complementation Tests of Enhancer Mutations

Crosses	En(var)KD201	En(var)KD202	En(var)KD203	En(var)KD204
En(var)KD201	0/471*	0/301	0/392	0/446
En(var)KD202		0/678	0/356	0/371
En(var)KD203			0/660	0/431
En(var)KD204				0/431

Notes

* A/B: A is the Cy^+ flies with genotype En(var)1/En(var)2. and B is the Cy flies with genotype En(var)1/CyO or En(var)2/CyO.

7. Effects of Su(var) or En(var) on Eye Pigment of w^{m4}

Visual examination indicated that all the suppressor mutations strongly increased the amount of eye pigment in w^{m4} flies, while the enhancer mutations reduced it. To quantify these effects, crosses were made between Su(var)/Balancer or En(var)/Balancer and w^{m4}; +/+ (#67). The progeny of these crosses were sorted into Su(var)/+ and Balancer/+ or En(var)/+ and Balancer/+ flies based on the dominant markers on the balancer chromosomes. Then the amount of eye pigment was measured for flies with different genotypes according to Materials and Methods 7.

There are two main eye pigments, red and brown. However, only the brown pigment was measured since modifier mutations affect the red and brown pigment proportionally, and the measurement of both pigments only produces duplicate results. Moreover, for unknown reasons, the brown pigment values, on average, are less variable than those for the red pigment (Locke *et al.*, 1988).

The pigment of Balancer/+ progeny served as a control. This control was required since the variegating phenotype of w^{m4} could be affected by many environmental and genetic background factors.

The measurements of amount of brown eye pigment in Su(var)/+ flies are shown in Table 8. In all 20 comparisons, the amount of eye pigment in Su(var)/+ progeny was significantly greater than that in the the Balancer/+ controls (see Table 8) in both males and females (all P-values are less than 0.001 according to the Student's t-test, see Materials and Methods 8). In all cases, a comparison of males and females for both Su(var)/+ and Balancer/+, produced values that are significantly different ($P < 0.01$).

For the 6 second chromosome suppressor mutations, each mutation has a different effect on the amount of eye pigment in w^{m4} progeny. For example, Su(var)KD206 and 208 are very strong suppressor mutations, and each can increase the eye pigment of w^{m4} flies variegation to values approximating wild type. Alternatively the Su(var)KD209 is a weak suppressor mutation. Flies from this strain have only about 1/7 as much pigment as Su(var)KD208 flies .

For third chromosome homozygous lethal mutations, members of complementation lethal group I, including Su(var)KD306, 310, 322, 328 and 340, each contain similar amounts of pigment. This is also true for two suppressor mutations Su(var)KD343 and 346, which belong to other complementation groups. On the other hand, flies from two other complementation groups (Su(var)KD334 and Su(var)KD341) have much lower pigment values than the above two complementation groups. These results suggest that the suppressing effects of members from the same lethal complementation group are approximately equal.

A visual examination indicated that the male eyes of all four enhancer mutants were completely white while female eyes

Notes of Table 8

a). SEM is Standard Error of the Mean of three samples.

b). Oregon-R is a wild-type *Drosophila melanogaster* stock.

c). The genotype of #27 is *w; Sb e P[ry+ Δ2-3](99B)/TM6,Ubx e* and the flies with this genotype have completely white eyes.

d). A w^{m4} stock, which is wild type for the second and third chromosomes.

e). 201, 202 etc. represent Su(var)KD201, Su(var)KD203 etc.

f). Su/+ represents chromosomes bearing a suppressor mutation.

g). Bal./+ is the balancer chromosome serving as a control, in that no suppressor mutations are present.

h). P is the P value according to the Student's t-test (see Materials and Methods section 8).

Asterisk (*) means that the variances are unequal. Therefore, the approximate P-values were calculated (see Materials and Methods section 8).

Table 8 Effects of suppressor mutations on amount of brown pigment in the eyes of w^{m4} flies

Su(var)KD Mutations	Absorbance ± SEM[a] Male			Absorbance ± SEM Female			Male/Female Pigment Ratio	
Oregon-R[b]	1.122 ± 0.017			1.372 ± 0.023				
w- (#227)[c]	0.004 ± 0.000			0.004 ± 0.004				
w^{m4}; +/+ (#67)[d]	0.036 ± 0.009			0.034 ± 0.004				
Alleles[e]	Su/+[f]	Bal/+[g]	P[h]	Su/+	Bal./+	P	Su/+	Bal./+
201	0.132± 0.021	0.015± 0.004	0.003	0.637± 0.060	0.148± 0.002	* 0.0073	0.207	0.101
203	0.180± 0.004	0.013± 0.002	< 0.0001	0.733± 0.013	0.142± 0.003	< 0.0001	0.246	0.092
208	0.729± 0.023	0.015± 0.002	* 0.0005	1.356± 0.028	0.045± 0.002	* 0.0002	0.538	0.333
209	0.107± 0.006	0.016± 0.001	* 0.0020	0.445± 0.091	0.069± 0.001	* 0.0104	0.240	0.232
211	0.292± 0.057	0.014± 0.000	* 0.0198	0.790± 0.11	0.045± 0.002	* 0.0096	0.370	0.311
206	0.520± 0.015	0.016± 0.001	* 0.0005	1.278± 0.036	0.042± 0.007	< 0.0001	0.407	0.381
335	0.046± 0.008	0.012± 0.003	0.0003	0.159± 0.012	0.057± 0.005	< 0.0001	0.289	0.211
336	0.319± 0.013	0.005± 0.001	* 0.0008	1.367± 0.013	0.025± 0.002	* 0.0001	0.233	0.200
337	0.204± 0.011	0.006± 0.001	* 0.0019	1.195± 0.017	0.025± 0.001	* 0.0001	0.171	0.240
338	0.355± 0.004	0.008± 0.000	* 0.0001	1.286± 0.050	0.051± 0.005	* 0.0008	0.276	0.157
354	0.301± 0.020	0.018± 0.004	* 0.0021	1.233± 0.035	0.069± 0.014	< 0.0001	0.244	0.261
306	0.270± 0.034	0.002± 0.002	* 0.0078	1.358± 0.060	0.043± 0.011	< 0.0001	0.199	0.047
310	0.257± 0.015	0.007± 0.000	* 0.0018	1.474± 0.008	0.031± 0.002	< 0.0001	0.174	0.226
322	0.213± 0.010	0.003± 0.001	* 0.0010	1.339± 0.016	0.025± 0.003	< 0.0001	0.159	0.120
328	0.291± 0.013	0.002± 0.000	* 0.0015	1.425± 0.010	0.030± 0.003	< 0.0001	0.204	0.067
340	0.315± 0.011	0.002± 0.001	* 0.0006	1.419± 0.001	0.029± 0.001	< 0.0001	0.222	0.069
334	0.054± 0.004	0.005± 0.001	0.0002	0.369± 0.005	0.019± 0.001	< 0.0001	0.146	0.263
341	0.064± 0.005	0.010± 0.001	* 0.0036	0.475± 0.064	0.018± 0.001	* 0.0095	0.135	0.556
343	0.457± 0.031	0.007± 0.001	* 0.0024	1.078± 0.030	0.028± 0.002	* 0.0004	0.424	0.250
346	0.343± 0.065	0.007± 0.0003	* 0.0188	1.083± 0.014	0.030± 0.007	< 0.0001	0.317	0.233

contained very little pigment. To quantify these effects, crosses were made between En(var)/CyO and w^{m4}; +/+ (#67) flies. The progeny of these crosses were sorted into En(var)/+ and CyO/+ flies based on the dominant marker Cy. Then the amount of brown eye pigment was measured. The results are shown in the Table 9. All four En(var)/+ flies contain less pigment compared to the control balancer/+ flies. The P-values are less than 0.001, except for En(var)KD201 females (the P-value is less than 0.05), according to the Student's t-test (see Materials and Methods 8).

Table 9

Effects of enhancer mutations on amount of brown pigment in the eyes of w^{m4} flies

	Absorbance ± SEM[a] Males			Absorbance ± SEM Females		
Mutations	En/+[b]	Bal./+[b]	P[c]	En/+	Bal/+.	P
En(var)KD201	0.0003 ±0.000	0.0087 ±0.000	< 0.0001	0.009 ±0.002	0.066 ±0.026	0,048
En(var)KD202	0.001 ±0.000	0.015 ±0.001	0.0002	0.004 ±0.001	0.201 ±0.015	0.0008*
En(var)KD203	0.001 ±0.000	0.015 ±0.001	0.0004	0.005 ± 0.0009	0.164 ±.015	0.0046*
En(var)KD204	0.004 ±0.001	0.018 ±0.000	< 0.0001	0.001 ±0.000	0.149 ±0.001	< 0.0001

Notes:

a. SEM is the Standard Error of the Mean of three samples.

b. En/+ represents chromosomes bearing enhancer mutation and Bal./+ is the balancer chromosome serving as a control.

c. P is the P value according to the Student's t-test (see Materials and Methods section 9). Asterisk (*) means that the variances are unequal. Therefore, the approximate P-values were caculated (see Materials and Methods section 8).

8. Effects of Su(var) and En(var) Mutations on B^{sv}

Previous studies showed that the mutation of modifier genes influence different variegating alleles (Sinclair *et al.*, 1983; 1989; Locke *et al.*, 1988) possibly because the modifier genes encode

proteins that are generally required for the formation of heterochromatin. To test whether suppressor and enhancer mutations described herein affect position effect variegation generally or act specifically on the variegating mutation w^{m4}, their effects on another variegating mutation, B^{sv}, were examined.

The dominant *Bar* mutation is a duplication of region 16A of the X-chromosome which results in narrow eyed flies (Sturtevant, 1925; Bridges; 1936). When Bar is translocated to the heterochromatic Y-chromosome, as in B^{sv}, the expression of Bar can be inactivated by the Y-chromosome heterochromatin, causing a shift in the genotype toward wild type. It was demonstrated that in B^{sv} flies the inactivation of Bar by Y-chromosome heterochromatin is a V-type position effect (Brosseau, 1960). Therefore, suppression of PEV by a Su(var) mutation will result in a stronger Bar phenotype (narrow eyes) while enhancement will result in a more wild type phenotype. In this test, virgin females of Su(var) or En(var) balanced by CyO or TM6 were crossed to *Inscy*/ B^{sv} *Y y+* (#228) males. From each cross, the eye width of 10 male progeny of each genotype were measured under a dissecting microscopy with an ocular micrometer.

The modifier effects are shown in Table 10. Compared to the control flies (balancer/+), all the Su(var)/+ flies suppress the B^{sv} toward the Bar phenotype ($P < 0.0001$). All enhancer mutations significantly enhance the inactivation of B^{sv} toward wild type ($P < 0.0001$). In contrast to the effect of suppressor mutations on w^{m4}, in which various mutations affect eye color to different strength, it seems that there is no significant difference between different

Table 10 The effect of Suppressor and Enhancer mutations on B^{sv}

Alleles	Width of Male Eyes ± SEM[a] (O. U.)		P-Values[b]	Su (var)/Bal. Eye Width
	Su(var)[c]/+	Bal.[d]/+		
Su(var)KD201	0.33 ± 0.01	0.59 ± 0.02	0.0001*	0.56
Su(var)KD203	0.41 ± 0.02	0.74 ± 0.02	<0.0001	0.55
Su(var)KD206	0.38 ± 0.02	0.77 ± 0.03	0.0001*	0.50
Su(var)KD208	0.40 ± 0.01	0.71 ± 0.04	0.0001*	0.56
Su(var)KD209	0.48 ± 0.02	0.85 ± 0.02	<0.0001	0.56
Su(var)KD211	0.38 ± 0.02	0.81 ± 0.03	0.0001*	0.47
Su(var)KD306	0.41 ± 0.02	0.84 ± 0.02	<0.0001	0.49
Su(var)KD310	0.54 ± 0.02	0.99 ± 0.01	0.0001*	0.55
Su(var)KD322	0.48 ± 0.02	0.91 ± 0.02	<0.0001	0.48
Su(var)KD328	0.36 ± 0.02	0.93 ± 0.02	<0.0001	0.40
Su(var)KD340	0.52 ± 0.02	0.92 ±0 .02	<0.0001	0.57
Su(var)KD334	0.45 ± 0.02	0.86 ± 0.02	<0.0001	0.52
Su(var)KD341	0.31 ± 0.01	0.82 ± 0.01	<0.0001	0.39
Su(var)KD343	0.36 ± 0.01	0.67 ± 0.02	0.0001*	0.54
Su(var)KD346	0.28 ± 0.01	0.44 ± 0.02	0.0001*	0.63
Su(var)KD335	0.32 ± 0.01	0.55 ± 0.03	0.0001*	0.58
Su(var)KD336	0.45 ± 0.02	0.96 ± 0.02	<0.0001	0.49
Su(var)KD337	0.43 ± 0.02	0.88 ± 0.02	<0.0001	0.49
Su(var)KD338	0.45 ± 0.01	0.76 ± 0.03	0.0001*	0.59
Su(var)KD354	0.44 ± 0.01	0.64 ± 0.03	0.0001*	0.68
	En(var)[e]/+	Bal.[d]/+		En(var)/Bal.
En(var)KD201	1.05 ± 0.02	0.67 ± 0.02	<0.0001	1.57
En(var)KD202	1.04 ± 0.01	0.70 ± 0.02	0.0001*	1.49
En(var)KD203	1.01 ± 0.01	0.62 ± 0.03	0.0001*	1.63
En(var)KD204	1.04 ± 0.03	0.65 ± 0.03	<0.0001	1.60

Notes

a. SEM represents Standard Error of the Mean of 20 eye measurements (10 flies), and 1.0 O.U. = 0.33 mm at 40 X magnification.

b. The P-values according to the Student' t-test. Asterisk (*) means that the variances are unequal. Therefore, the approximate P-values were caculated (see Materials and Methods section 8)

c. Su(var)/+ represents the chromosome bearing Su(var)/+ mutations.

d. Bal./+ is the balancer chromosome serving as control.

e. En(var)/+ represents the chromosome having En(var) mutations.

suppressor mutations on their effects on the B^{sv} compared to Balancer/+ controls.

9. Recombination Mapping of Su(var)KD306, 328, 340 and En(var)KD201

The large number of Su(var) mutations recovered made it impractical to genetically map all of the mutations. Consequently, only 3 suppressor and one enhancer mutations were mapped. Su(var)KD306, 328 and 340 were chosen because 1). they belong to the same lethal complementation group. 2). They are very strong suppressor mutations. and 3). The Southern analyses revealed that there was an intact pUChsneo P-element present in the genome of Su(var)KD340.

These mutations were mapped by standard mapping procedures as described in Materials and Methods section 9 and Figure 11.

The Standard Error (SE) was calculated according to the following formula: $SE = \sqrt{P(1-P)/N}$, where $P = n/N$, n is the number of crossing over between marker gene and the mutation mapped, and N is the total number of flies scored (Ashburner, 1989).

Table 11

Recombination mapping of Su(var)KD306, 328, 340 and En(var)KD201

Mutations mapped	Genetic position	Reference locus	Frequency[a]	Possible cytogenetic locus
Su(var)KD306	57.9 ± 0.2	*Sb* (3-58.2)	5/1552	87A-89A[b]
Su(var)KD328	57.3 ± 0.3	*Sb* (3-58.2)	13/1410	87A-89A
Su(var)KD340	55.6 ± 1.1	*Sb* (3-58.2)	38/1487	87A-89A
En(var)KD201	17.4 ± 1.0	*dp* (2-13.0)	20/458	24A-30E[c]

Notes:

a. Frequency = Recombinants/Total flies scored.

b. These mutations were mapped between *Gl* (3-41.4) and *Sb* (3-58.2, 89B-90A) and very close to *Sb*. The possible cytogenetic location was estimated from these two markers.

c. This mutation was mapped between *dp* (2-13.0, 24E2-25A2) and *b* (2-45.8, 34E2-35D1) but closer to *dp*. The possible cytogenetical location was estimated from these two markers.

For calculating the map position of these three mutations, the *Sb* (3-58.2, 89B9-10) marker was used as a reference point since these mutations are relatively closer to the *Sb*. As expected from the complementation test results, they all were mapped to the same chromosome region (Table 11).

The En(var)KD201 was mapped to 17.4 ± 1.0 of the second chromosome by using *dp* (2-13.0) as a reference point since *dumpy* is closer to the En(var)KD201 than *black* (*b*).

10. Cytogenetic Analyses of Suppressor Mutations

To determine whether there were any chromosomal aberrations associated with the Su(var) and En(var)-bearing chromosomes, the polytene chromosomes of heterozygotes Su(var)/+ and En(var)/+ were prepared and examined as described in the Materials and Methods 10. These results are summarized in Table 12 and Figure 14.

Figure 14. Salivary gland polytene chromosomes from Su(var)KD201/+ (A), En(var)KD201/+ (B), Su(var)KD306/+ (C), 328/+ (D) and 340/+ (E).

The En(var)KD201 derived from Su(var)KD201 was mapped at 17.0±1.0 to the right of *dp* (2-13.0, 24E2-25A2). This corresponds to the polytene chromosome region 25A-27A. Chromosomes of Su(var)KD201(A) and En(var)KD201 (B) show no visible chromosomal aberrations between the 25A-27A regions.

Su(var)KD306, 328 and 340 were mapped to a region just left of *Sb* (3-58.2, 89B9-10). Chromosomes of Su(var)KD306 (C), Su(var)KD328(D) and Su(var)KD340(E) show no visible chromosomal aberration between the 88A-90A regions.

The polytene chromosomes were prepared and examined as described in Materials and Methods 11 (Mag. ≈1750X).


A
27A
26A
25A
27A
26A
25A
B
C
88A
89A
90A
D
89A
88A
E
89A
88A

For the unmapped second chromosome mutations (Su(var)KD203, 206, 208, 209 and 211), both the 2L and 2R regions of polytene chromosomes were examined carefully, and no chromosomal aberrations were observed.

The En(var)KD201 mutation was genetically mapped to 17±1.0 of the second chromosome between *dp* (24E2-25A2) and *b* (34E2-35D1). The intervening polytene chromosomal region (24A-35D1) of this mutation was carefully examined, but no visible aberration was found (Figure 14 B). When the same region of Su(var)KD201 (the parental stock) was carefully examined, again no chromosome aberration was observed either (Figure 14 A).

For the unmapped third chromosome mutants, Su(var)KD310, 322, 324, 335, 336, 337, 338 346, careful examination of polytene chromosome arms 3L and 3R for each of the mutations showed no visible chromosome aberrations. The three alleles of the same lethal complementation group (Su(var)KD306, 328 and 340) were mapped to a region very close to *Sb* (3-58.2; 89B9-10) between *Gl* (3-41.4) and *Sb*. The intervening region of the polytene chromosome of these mutations was carefully examined (Figure 14 C, D and E), and no visible aberration was observed.

Table 12

Cytogenetic studies of suppressor and enhancer mutations

Mutations	#. of Slides Examined	#. of Nuclei Examined	Chromosome Arms Examined	Regions* Examined in Detained	Observed Aberrations
En(var)KD201	3	16	2L and 2R	24A - 30E	0
Su(var)KD201	2	15	2L and 2R	24A - 30E	0
Su(var)KD203	2	11	2L and 2R		0
Su(var)KD206	2	12	2L and 2R		0
Su(var)KD208	2	11	2L and 2R		0
Su(var)KD209	2	14	2L and 2R		0
Su(var)KD211	1	10	2L and 2R		0
Su(var)KD306	3	20	3L and 3R	87A - 89A	0
Su(var)KD310	2	12	3L and 3R		0
Su(var)KD322	3	12	3L and 3R		0
Su(var)KD328	3	11	3L and 3R	87A - 89A	0
Su(var)KD340	3	12	3L and 3R	87A - 89A	0
Su(var)KD334	3	14	3L and 3R		0
Su(var)KD335	2	17	3L and 3R		0
Su(var)KD336	2	10	3L and 3R		0
Su(var)KD337	2	11	3L and 3R		0
Su(var)KD338	1	12	3L and 3R		0
Su(var)KD346	2	22	3L and 3R		0
Su(var)KD341	3	17	3L and 3R		16 (95%)

Notes:
* These regions examined in detail were determined from the recombination studies based on the cytogenetic positions of two markers between which the mutations were mapped (see Results section 10).

11. Cytogenetic Analyses of Su(var)KD341

Initial examination of the polytene chromosomes of Su(var)KD341 showed that the tips of 3L and 3R were attached together 95% (16/17) of the time. Therefore, they would form a "ring chromosome". The typical attachments of 3L and 3R tips are shown in the Figure 15 A, B, C. Careful examinations of 3L and 3R tips revealed no visible change in their banding patterns (Figure 15 B and D). In the one nucleus (among 17 nuclei) in which the 3L and 3R were not attached, the banding patterns of the 3L and 3R chromosome tips appeared normal (see Figure 15 C).

The observation of attached 3L and 3R tips is unusual and required further attention. Is this attachment unique to Su(var)KD341 mutation stock, caused by the suppressing effect, or it is just caused by some preparation artifact(s)?

To answer this question, polytene chromosomes were again prepared but with some controls as well. The Oregon-R chromosomes were used as a control to rule out the possibility that the attachment of 3L and 3R tips is caused by some preparation artifact. Since an initial examination of the polytene chromosomes of the other suppressor and enhancer mutations revealed no attachment of 3L and 3R tips, it seems that the attachment of 3L and 3R is not a general property of suppressor or enhancer mutations. It is particularly associated with the third chromosome of the Su(var)KD341 mutation. To confirm these observations, the Su(var)KD340 mutation was used as another control. Virgin females of wild type (Oregon-R) were crossed to Su(var)KD341, Su(var)KD340 and wild type (Oregon-R) males, respectively. Then the polytene chromosomes from F_1 larvae of these three crosses were prepared at the same time, using the same chemicals and procedures. The results in Table 13 show that only the polytene chromosomes of Su(var)341/+ had attached 3L and 3R tips at a frequency of 94%. The frequency of 2L and 2R attachment in all these three crosses and the 3R and 3L attachment in Su(var)KD340/+ and wild type (Oregon-R) larval polytene chromosomes is 0%.

The above result suggest that the high frequency of the attachment of 3L and 3R tips is associated with the third chromosome of the Su(var)KD341 stock. It is possible that the

Figure 15. Salivary gland polytene chromosomes from Su(var)KD341/+ showing the attachment of 3L and 3R tips.

A. shows that 3L and 3R tips attached to form a "ring".

B. and D. show the typical attachment of 3L and 3R tips.

C. shows a rare unattached 3L and 3R from one nucleus of Su(var)KD341/+. CC is the chromocenter.

The polytene chromosomes were prepared and examined as described in Materials and Methods 11 (Mag. ≈1500X).


CC
3L
B
3L
3L
2R
2R

attachment of 3L and 3R is due to the "ectopic pairing" of the 3L and 3R tips, and is not caused by any chromosomal rearrangment (inversion and translocation). However, conclusions can not yet be made regarding whether this attachment is really "ectopic pairing" or due to chromosomal aberrations, and whether it is caused by the Su(var)KD341 mutation or other genetic factors.

Table 13

Frequency of attachment of the polytene chromosome tips in Su(var)KD341

Stocks[a]	# of Slides Examined	# of Nuclei Examined	2L and 2R tips attached[b]	3L and 3R tips attached[b]
Oregon-R	3	37	0 (0%)	0 (0%)
Su(var)KD340	2	21	0 (0%)	0 (0%)
Su(var)KD341	2	47	0 (0%)	44 (94%)

Notes:

a). Oregon-R is a wild type *Drosophila melanogaster* stock. Su(var)KD340 is another suppressor mutation stock. Both these stocks served as controls.

b). % = Number of nuclei showing attachment of the polytene chromosome tips/Number of nuclei examined.

12. Interaction Between En(var) and Su(var) Mutations

During the screening for suppressor mutations, four enhancer mutations were derived from unstable suppressor mutation stocks. To test the phenotypic interaction between these suppressor and enhancer mutation pairs, crosses were made between Su(var)/CyO and En(var)/CyO stocks. The eye pigment of progeny En(var)/CyO, Su(var)/CyO and Su(var)/En(var) flies were measured. The results are shown in Table 14.

Table 14

Phenotypic interaction between enhancer and suppressor mutations

		Absorbance ± SEM[a]				
Crosses	Sex	En/CyO[b]	En/Su[c]	Su/CyO[d]	P1[e]	P2[e]
En(var)201 X	M	0.007±0.000	0.025±0.002	0.249±0.026	0.0001	0.006*
Su(var)201	F	0.007±0.001	0.246±0.020	0.910±0.035	0.004*	<0.0001
En(var)202 X	M	0.003±0.000	0.047±0.003	0.256±0.003	0.0001	0.002*
Su(var)202	F	0.010±0.001	0.223±0.014	1.013±0.014	0.003*	<0.0001
En(var)203 X	M	0.007±0.000	0.029±0.001	0.293±0.010	<0.0001	0.0007*
Su(var)203	F	0.009±0.000	0.238±0.025	0.238±0.025	0.006*	<0.0001
En(var)204 X	M	0.007±0.001	0.019±0.000	0.186±0.004	<0.0001	0.0006*
Su(var)209	F	0.009±0.000	0.178±0.002	0.845±0.024	<0.0001	0.0002*

Notes:

a. SEM represents the Standard Error of the Mean of three samples.

b. En/CyO are the chromosomes bearing the En(var) mutation/balancer CyO.

c. En/Su are the chromosomes bearing the En(var) mutation/the chromosome bearing Su(var) mutation.

d. Su/CyO are the chromosome having the Su(var) mutation/balancer CyO.

e. P1 and P2 are the P-values according to the Student's t-test. P1 is the comparison between En/CyO and En/Su, while the P2 is the comparasion of En/Su to Su/CyO.

Asterisk (*) means that the variances are unequal. Therefore, the approximate P-values were caculated (see Materials and Methods section 8).

In all four En(var) and Su(var) combinations, the pigment values of En(var)/Su(var) flies are significantly greater than En(var)/CyO flies (P1 < 0.001), but significant less than Su(var)/CyO flies (P2 < 0.001) for both males and females. The pigment levels of all four pairs of Su(var)/En(var) double mutants differed substantially from both single Su(var) and En(var) mutations. The enhancer mutations can reduce the suppression effect of Su(var) mutation or vice versa. Thus the phenotypic interaction between these Su(var) and En(var) mutations appears to be additive and not epistatic.

13. Genomic Southern Analyses

All the suppressor mutations were induced in flies containing pUChsneo and Δ2-3 P-elements. This should lead to the mobilization of the pUChsneo P-element, and the reinsertion of a pUChsneo P-element to cause the mutations recovered. To test whether there is an intact pUChsneo P-element or other P-element sequences present in the genome of each suppressor mutation, the genomic DNA of 20 independently-isolated suppressor mutants was analysed by probing Southern blots with pUC19N DNA and P-element sequences. To detect an inserted pUChsneo P-element, pUC19N was used to detect the plasmid portion (pUC19N is derived from pUC8 but has a different polylinker). The P-element portion was detected using two fragments isolated from *Bam*HI-*Sal*I double digestion of pπ25.1 as probes (see Figure 1 C). This DNA fragment is about 4.3 kb long and contains Drosophila genomic DNA (form 17C) plus a complete 2.9 kb P-element which has homology with both ends of the pUChsneo P-element (Steller and Pirrotta, 1985).

Genomic DNAs were prepared as described in Materials and Methods section 12. Five μg of genomic DNA, digested with with *Eco*RI, or *Eco*RI and *Xho*I, were separated in each lane and capillary blotted onto GeneScreen Plus membranes. These membranes were then hybridized with probes labeled with [α-^{32}p] dCTP as described in Materials and Methods section 11. The results of genomic Southern blot analyses of the Su(var)KD206, 306, 310, 328 and 340 are shown in Figure 16. The results of genomic Southern analysis of other suppressor mutations are not shown.

In the pUChsneo P-element polylinker, there is an EcoRI site (see Figure 1 B). When the genomic DNA was cut with EcoRI and then probed with pUC19N, each intact pUChsneo P-element present in the genome should produce two bands on the southern blot: (1) a dense band corresponding to a DNA fragment showing about 2.5 kb homology to pUC19N plasmid. and (2) a faint band corresponding to a DNA fragment containing only 170 bp of pUC19N sequence. The size of these two DNA fragments depends on the adjacent *Eco*RI site in the genomic DNA.

When the genomic DNA was double-digested with *Xho*I and *EcoR*I, two bands should also be expected; a dense band, about 2.5 kb in size, corresponding to the internal *Xho*I-*Eco*RI pUC8 sequence of pUChsneo P-element, and a fainter band, which may have the same size as in the single digestion with *Eco*RI (see Figure 1, the restriction map of pUChsneo P-element). The use the of the entire P-element should identify fragments containing the left end of a pUChsneo P-element sequence and probably any other P-elements present in the genome.

The genomic DNA of Canton-S (wild type, M-strain), Oregon-R (wild type, M-strain), π (wild type, P-strain with about 50 P-elements), *P[pUChsneo](9C); mwh red e* (#26) providing pUChsneo P-element and $ry^{506}P[ry^{+}\Delta 2\text{-}3](99B)$ (#39) having a transposase-producing P-element served as controls. As expected, in lane 10 having genomic DNA of *P[pUChsneo](9C); mwh red e* (#26) double-digested with EcoRI and XhoI and probed with pUC19N DNA, two bands were detected: one dense band corresponding to a 2.5 kb DNA fragment and one faint band corresponding to a 5 kb DNA sequence.

From some of the controls (such as Oregon-R, π, Canton-S) and some mutants, a DNA fragment about 5 kb long was detected. Since all of these fragments have the same size, they are probably due to the contamination of genomic DNA by some plasmid, with homology with pUC19N.

Su(var)KD340 DNA contained an intact pUChsneo P-element sequence. When this DNA was digested with *Eco*RI and then probed with pUC19N (Lane 9), two DNA fragments were found to hybridize with pUC19N. One is about 0.8 kb long, and another is about 6.5 kb in length. When the genomic DNA of Su(var)KD340 was doubly digested with *Eco*RI and *Xho*I (Lane 16), two bands were detected. One is a dense band corresponding to a DNA fragment about 2.5 kb long and the same size as the dense band detected in the parental *P[pUChsneo](9C); mwh red e* (#26) double digestion. The other is a faint band having the same size as when Su(var)KD340 DNA was digested with *Eco*RI alone. In addition, when the Su(var)KD340 genomic DNA was digested with *Sal*I, *Xho*I or *Bam*HI, different size of DNA fragments were detected, indicating that the plasmid rescuable fragments from Su(var)KD340 genome are different when different enzymes are used.

When the Su(var)KD328 genomic DNA was doubly digested with *Eco*RI and *Xho*I and probed with pUC19N (Lane 7), a single band corresponding to a 7 kb DNA fragment was observed. These results indicate that there is an incomplete pUChsneo P-element sequence present in the genome of Su(var)KD328.

In other mutations tested (Su(var)KD201, 203, 206, 208, 209, 211, 306, 310, 322, 334, 341, 335, 336, 337, 338, 342, 246, 354), no

pUC19N sequence was detected (Results not shown). It is very likely that these mutations were not caused by the insertion of a pUChsneo P-element. Plasmid rescue can not be used to clone these suppressor genes.

When the blots were stripped of the pUC19N probe, and then reprobed with the P-element fragment. None of the second chromosome suppressor mutations hybridized to this P-element sequence, indicating that no P-elements are present in these stocks (not shown). In the third chromosome suppressor mutations, many P-element sequences were detected (bottom panel). Most of the bands show the same size and are probably from the balancer chromosome, since no P-element sequences were detected in the second chromosome suppressor mutants, which have the same second and third chromosomes originating from *P[pUChsneo](9C); mwh red e* (#26) stocks. In the control lane containing genomic DNA of P-strain π, many P-elements were detected. In all lanes digested with *Eco*RI of Southern blots probed with P-element sequences (bottom panel), there is a dense hybridization band corresponding to a 9.4 kb DNA fragment. This band are due to the genomic DNA sequence present in the P-element probes isolated from pπ25.1.

In summary, the Southern blotting results indicated that there is an intact pUChsneo P-element sequence present in the genome of Su(var)KD340 and an incomplete pUChsneo P-element sequence in the Su(var)KD328. No pUChsneo P-element sequence was detected in the other suppressor mutations. No P-element sequences are present in the second chromosome suppressor mutations. On the other hand, several P-element sequences were detected in the third

Figure 16. Genomic Southern blot analyses of the Su(var)KD206, 306, 310, 328 and 340 mutations. Autoradiograph of genomic southern blot hybridized with pUC19N is as described in Materials and Methods 11 (Top). Autoradiograph of the same blot rehybridized with P-element sequences isolated from *BamHI and XhoI* double-digestion of pπ25.1 after the pUC19N probe was removed (Bottom).

Laneλ. λ HindIII fragments used as size markers.

The genomic DNAs below were digested with *Eco*RI.

Lane 1. Oregon-R (wild type, M strain)

Lane 2. π (wild type, P strain)

Lane 3. Canton-S (wild type, M strain)

Lane 4. $ry^{506}P[ry^{+}\Delta 2\text{-}3](99B)$ (#39)

Lane 5. *P[pUChsneo](9C); mwh red e* (#26)

Lane 6. Su(var)KD306

Lane 7. Su(var)KD328

Lane 8. Su(var)KD206

Lane 9. Su(var)KD340

The genomic DNAs below were digested with *Eco*RI/*Xho*I.

Lane 10. *P[pUChsneo](9C); mwh red e* (#26)

Lane 11. Su(var)KD306

Lane 12. Su(var)KD310

Lane 13. Su(var)KD322

Lane 14. Su(var)KD328

Lane 15. Su(var)KD206

Lane 16. Su(var)KD340

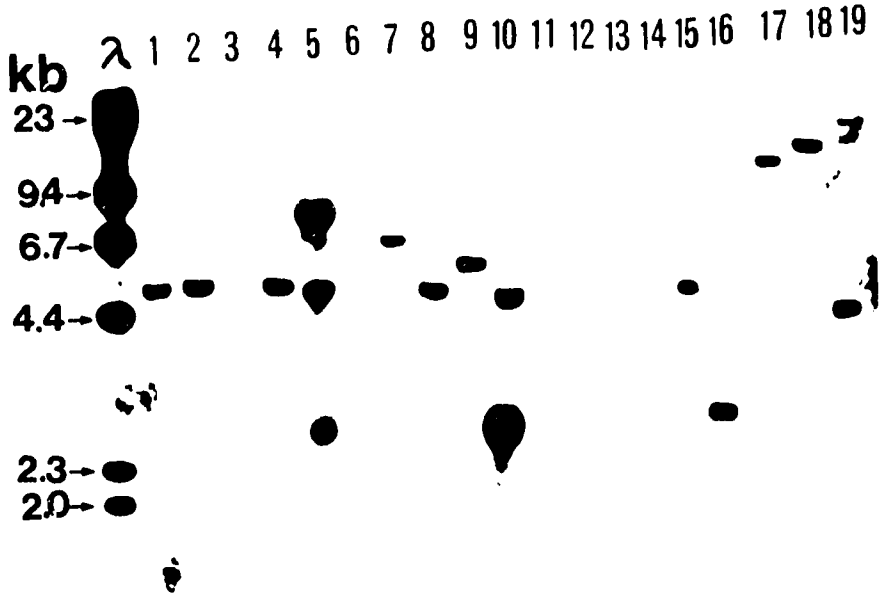
Lane 17. Su(var)KD340 digested with *Sal*I

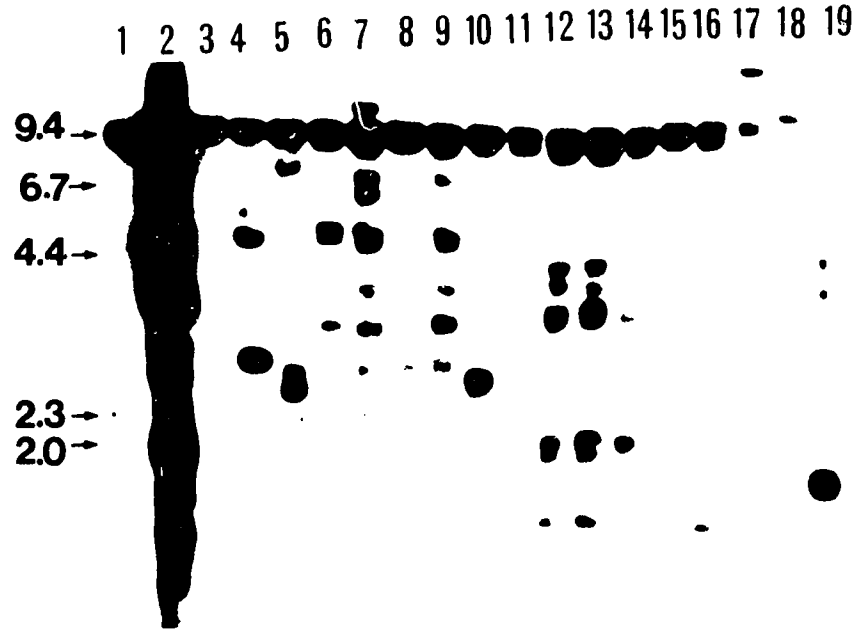
Lane 18. Su(var)KD340 digested with *Xho*I

Lane 19. Su(var)KD340 digested with *Bam*HI

The explanation of these results was shown in Results section14.

The genotype of each Su(var) is actually w^{m4}; *Su(var)/Balancer* . The second chromosome balancer is CyO and the third chromosome balancer is TM6.


kb
λ 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
23
9.4
6.7
4.4
2.3
2.0


1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
9.4
6.7
4.4
2.3
2.0

chromosome suppressor mutant genome, and most of them are presumably on the TM2 balancer chromosome.

14. Effects of Different Y-chromosomes on w^{m4}

Initially, all of the third chromosome suppressor mutations were kept with both CyO and TM2 balancer chromosomes, with the genotype w^{m4}; *+/CyO; mwh red e*/TM2, Ubx e.* Soon it was realized that it was impossible to remove the CyO balancer chromosomes from these stocks by simple sib-mating since the sib-crosses of w^{m4}; *+/CyO; mwh red e*/TM2, Ubx e* could not produce any progeny. It is possible that the second chromosome causes sterility when homozygous. To replace these second chromosomes, virgin females of w^{m4}; *+/CyO; mwh red e*/TM2, Ubx e* were crossed to w^{m4}; *MRS/TM6, Ubx e* (#8) males. The male progeny of these crosses showed w^{m4} phenotype, whereas all the females remained fully suppressed (near wild type eye pigment). For example, the male eyes of Su(var)KD328 contain very little pigment (0.065), while the female eyes have much more pigment (1.224, see Table 15).

Moreover, from one line, called Su(var)KD328(A), a single male with a strong suppressor phenotype was found. When this male was crossed to Su(var)KD328(A) females, another line, called Su(var)KD328(B), was established. There is no significant female eye pigment difference between these two lines of Su(var)KD328 mutations (1.244 vs 1.369). On the other hand, the males of Su(var)KD328(B) have much more eye pigment than that of Su(var)KD328(A) (0.918 vs 0.065).

What caused the Su(var)KD328(A) males to revert to a typical w^{m4} phenotype? What made the Su(var)KD328(B) males different from those of Su(var)KD328(A)? To answer these questions, reciprocal crosses were made among Su(var)KD328(A), w^{m4}, +/+ (#67) and Su(var)KD328(B) flies. The progeny were sorted into males and females. Since visual examination revealed no eye pigment difference among the flies with different genotypes, the flies were not sorted into different genotypes. Then the brown pigment was measured in these flies according to Materials and Methods section 7.

The results of these crosses are shown in Table 15. All are consistent with the view that the Y-chromosome is responsible for the difference between Su(var)KD328(A) and Su(var)KD328(B) males. In reciprocal crosses between Su(var)KD328(A) and Su(var)KD328(B) lines, there is no significant difference in the eye pigment of female progeny (1.454 vs 1.226). However, the male progeny resemble their fathers in that they have more pigment if the Y-chromosome came from Su(var)KD328(B) males (0.057 vs 0.704).

The reciprocal crosses between Su(var)KD328(A) and w^{m4}; +/+ (#67) flies showed that the male progeny from w^{m4}; +/+ (#67) fathers contain more pigment (1.189 vs 0.290). However, in similar crosses with Su(var)KD328(B), no significant difference in eye pigment among male progeny was observed (1.169 vs 1.157).

From these observations, it can be concluded that there must be some difference between the Y-chromosomes of Su(var)KD328(A) and Su(var)KD328(B), and no significant difference between

Su(var)KD328(B) and w^{m4}, +/+ (#67) Y-chromosomes in their effects on the w^{m4} variegation. Different Y-chromosomes have different effects on the expression of w^{m4} in the suppressor mutations.

It should be pointed out that the male specific suppression effect seen in the Su(var)KD328(B) line was not due to an extra Y-chromosome because the male progeny from a cross between Su(var)KD328(B) males and *XX*, w^{48h} (#16) virgin females were sterile. Had the suppression been due to an extra Y-chromosome, these males could be fertile. This is a way to test whether the suppression is due to an extra Y-chromosome (see Materials and Methods 5 for more explanation).

Table 15

Effect of different Y-chromosomes on the expression of suppressor mutations

Su mutations or crosses Males	Females	Absorbance ± SEM[a] (Males)	P[b]	Absorbance ± SEM (Females)	P
Su(var)328(A) line[c]		0.065±0.006	<0.0001	1.244±0.014	0.003
Su(var)328(B) line[c]		0.918±0.008		1.369±0.021	
w^{m4}; +/+ (#67)		0.062±0.009		0.034±0.004	
Male Parent	Female parent				
Su(var)328(A)	Su(var)328(B)	0.057±0.005	<0.0001	1.454±0.030	0.40
Su(var)328(B)	Su(var)328(A)	0.704±0.029		1.226±0.068	
Su(var)328(A)	*w*^{m4}; +/+(#67)	0.290±0.004	0.0009*	1.537±0.003	0.057
w^{m4}; +/+(#67)	Su(var)328(A)	1.189±0.039		1.546±0.004	
Su(var)328(B)	*w*^{m4}; +/+(#67)	1.169±0.001	0.2649*	1.408±0.029	0.040
w^{m4}; +/+(#67)	Su(var)328(B)	1.154±0.019		1.495±0.023	

Notes
a. SEM is the Standard Error Mean of three samples.
b. P is the P-values according to the Student's t-test.
c. The Su(var)KD328(B) line was derived from the Su(var)KD328(A) line. The males of the Su(var)KD328(B) line have more eye pigment than those of the Su(var)KD328(A) line.
Asterisk (*) means that the variances are unequal. Therefore, the approximate P-values were caculated (see Materials and Methods section 8).

15. Effects of Parental Sources of *w*^{m4} on the PEV

A survey of eye pigment values in males and females of several *w*^{m4} stocks from our lab showed that males and females of the same stock have different amounts of eye pigment of (see the Table 16). For example in the *w*^{m4}, +/+ (#67) stock, females have less eye pigment than males (females 0.034 vs males 0.062). However, in other stocks (*w*^{m4}; *MRS/TM6, Ubx e* (#8) and *w*^{m4}; *CyO/Xa/TM2, Ubx e* (#58)) females have more pigment than males (females 0.113 vs males 0.016 in #8 and females 0.573 vs males 0.112 in #58). Initially I thought that these differences were probably caused by Y-chromosome differences among these stocks.

To test this hypothesis, reciprocal crosses were made among these three stocks, and then the eye pigment of male and female progeny was measured. The results are shown in Table 16. Visual examination indicated that all progeny of the same sex had a similar w^{m4} eye phenotype, regardless of the mix of genotypes. Therefore, the same sex progeny of all genotypes were assayed together for eye pigment. In wild type Oregon-R flies, the females contain a little more eye pigment than males (females 1.327 vs males 1.122), which is because female eyes are slightly larger than the male's.

Table 16

Effect of parental source of w^{m4} on the fly eye pigment

w^{m4} stocks and crosses Males	Females	Absorbance ± SEM[a] (Males)	P[b]	Absorbance ± SEM (Females)	P[b]
w^{m4}; +/+ (#67)		0.062±0.009		0.034±0.004	0.024
w^{m4}; *MRS/TM6* (#8)		0.016±0.002		0.113±0.023	0.0268*
w^{m4}; *CyO/Xa/TM2* (#58)		0.112±0.015		0.573±0.014	<0.0001
Oregon-R (wild type)		1.122±0.017		1.327±0.023	
Male parent	Female parent				
#67	#58	0.449±0.028	0.0027*	0.444±0.018	0.0001
#58	#67	0.045±0.002		0.127±0.020	
#8	#58	0.061±0.002	0.0001	0.275±0.029	0.0026
#58	#8	0.022±0.002		0.110±0.007	
#67	#8	0.060±0.037	0.0075*	0.120±0.014	0.0130*
#8	#67	0.023±0.001		0.034±0.001	

Notes
a. SEM is the Standard Error Mean of three samples.
b. P is the P-values according to Student's t-test.
Asterisk (*) means that the variances are unequal. Therefore, the approximate P-values were caculated (see Materials and Methods section 8).

In all three sets of reciprocal crosses, the progeny with the same genotype from a reciprocal cross expressed a different

phenotype for w^{m4} variegation. Both male and female progeny from a reciprocal cross contain significantly different eye pigments ($P < 0.05$ according to Student's t-test). In the reciprocal crosses between w^{m4}; +/+ (#67) and w^{m4}; *CyO/Xa/TM2, Ubx e* (#58), the cross with w^{m4}; *CyO/Xa/TM2, Ubx e* (#58) mothers and w^{m4}; +/+ (#67) fathers produced male progeny having about 10 times more eye pigment than the males from the reciprocal cross (0.449 vs 0.045), and the female progeny have about 4 times more (0.444 vs 0.127).

In another reciprocal cross involving w^{m4}; *MRS/TM2, Ubx e* (#58) and w^{m4}; *MRS/TM6, Ubx e* (#8), the cross with w^{m4}; *MRS/TM2, Ubx e* (#58) m thers also gave rise to progeny with higher pigment value than the corresponding reciprocal cross, males having about 3 times more (0.061 vs 0.022) and females having about 2 times more (0.275 vs 0.110).

The reciprocal crosses between w^{m4}; +/+ (#67) and w^{m4}; *MRS/TM6, Ubx e* (#8) also showed that in the cross with w^{m4}; *MRS/TM6, Ubx e* (#8) mothers, the progeny have higher pigment value than the cross with w^{m4}; +/+ (#67) mothers (0.060 vs 0.023 in males and 0.120 vs 0.034 in females). The difference in male eye pigment from reciprocal crosses can be partially accounted for by different Y-chromosomes. However, differences in the female progeny from the reciprocal crosses can not be explained this way since the female progeny should have the same genotype and consequently the same phenotype. The results from these experiments indicate that the parental contribution of w^{m4} must have effects on the expression of w^{m4} variegation in their progeny.

It should be pointed out that the different results from the reciprocal crosses were not due to environmental or developmental timing effects since all of these crosses were set up at the same time and cultured under the same conditions. Moreover, all the progeny from each cross were collected so that there is no collecting bias. When measuring the pigment, the head of flies were randomly picked from each group.

IV. Conclusions and Discussion

1. Characteristics of Su(var) and En(var) mutations

By using a pUChsneo P-element in combination with a stable transposase-producing Δ2-3 P-element as a "mutagen", 66 dominant suppressor mutations, representing at least 30 independently isolated mutations, were induced. Genetical studies of 20 independently isolated mutations showed that 6 of them were located on the second chromosome while 14 were located on the third chromosome. Like the previously studied Su(var) and En(var) mutations (Sinclair *et al.*, 1983; Locke *et al.*, 1988; Sinclair *et al.*, 1989), all of the suppressor mutations probably have a general effect on position effect variegation since they can suppress two different variegating alleles (w^{m4} *and* B^{sv}), that involve different regions of heterochromatin. However, it seems that these suppressor mutations affect the two variegating alleles differently. Different suppressor mutations can affect (increase) the w^{m4} eye pigment to different degree. Some suppressor mutations, such as Su(var)KD206, 208, 306, 310, 322, 328 and 340 are very strong suppressor mutations. These mutations can increase fly eye pigment of the w^{m4} variegation close to wild type levels. However, some mutations showed relatively weak effects on w^{m4}, such as Su(var)KD201, 203, 209, 211 and 335. In contrast, when all of these suppressor mutations were tested on their effects on another variegating allele (B^{sv}), it seems that there is no significant difference between different mutants with respect to their effects on the width of B^{sv} fly eyes. All of the suppressor

mutations decreased the width of eye to about half of the control *Balancer/+* flies.

This could be explained by the different levels of sensitivity of phenotypic assays with w^{m4} compared to those of B^{sv}. Under the pigment measurement condition used herein, the average eye pigment value of 10 wild type flies is about 1.4, while white flies with completely white eyes have an eye pigment value of 0. The strong suppressor mutations can suppress (increase) the eye pigment of w^{m4} flies to wild type level, whereas the strong enhancer mutations can enhance (decrease) the eye pigment of w^{m4} flies close to that measured in *white* flies. The relatively weak suppressor and enhancer mutations will produce flies with pigment values between 0 and 1.4. Thus variegating effects of different suppressor and enhancer mutations can be distinguished relatively well by using w^{m4} variegation. On the other hand, B^{sv} variegation can only give a resolution in the phenotypic assays ranging from about 0.3 (Bar mutations) to1.0 (wild type) under our measurement conditions. Within these limits the different effective of Su(var) mutations can not be distinguished.

Previous studies have shown that most of the suppressor and enhancer mutations of PEV were recessive lethals, and those which were recessive viable were often recessive steriles (Sinclair *et al.*, 1983; Reuter *et al.*, 1986; Locke *et al.*, 1988). These results suggested that most modifier genes have essential genetic functions. Consistent with the previous studies, most of suppressor mutations isolated in my studies are either recessive lethal (10/20 = 50%), or

recessive semi-lethal (6/20 = 30%), Those that were homozygous viable are generally recessively sterile for males and (or) females.

It should be pointed out that at this time we are still not sure if these lethal or sterile effects are caused by the Su(var) and En(var) mutations, or if they are due to second site mutations. Within limits in only one case, Su(var)KD340, the recessive lethality was linked to the suppressor mutation. However, even for this mutation, a definite conclusion can not yet be made regarding the association of the suppressor phenotype with lethality. To confirm this association, more experiments are needed. For example, if revertants could be recovered from these mutations, the concomitant loss of the recessive lethality in several revertants would indicate that the recessive lethality is a pleiotropic effect of the mutations. In addition, if some of the genes can be cloned and reintroduced into the Drosophila genome by P-element mediated transformation, their ability to rescue the lethality of the mutant could be determined directly.

For the 9 third chromosome recessive lethal mutations, complementation analyses identified four lethal complementation groups. Group I contained five independently isolated mutations, Su(var)KD306, 310, 322, 328 and 340. These mutations have the similar phenotypes. They suppress w^{m4} and B^{sv} to a similar degree. Genetic mapping of Su(var)KD306, 328 and 340 localized these three mutations to the same chromosome region (85A-90A). Previous studies have also identified several suppressor mutations in this region on the third chromosome (Sinclair *et al.*, 1983; Locke *et al.*, 1988). All of these results suggest that these mutations are probably

allelic. Groups II and III each contain a single mutation; Su(var)KD334 and Su(var)KD341 respectively. Complementation group IV consists of two mutations, Su(var)KD343 and 345. These two mutations suppressed w^{m4} to the same degree and both of them are lethal over TM2 or TM6, indicating that they share a lethal with these balancer chromosomes.

The lethality of one suppressor or enhancer mutation over another mutation in the same complementation group is unlikely to be caused by the synthenic lethality of the two mutations. As far as I know, no synthetic lethality of PEV modifier genes has been reported. From the complementation analyses (see Table 6), it was clear that two suppressor mutations from different lethal complementation groups can be viable. Those that were inviable (belonging to the same lethal complementation group) could be genetically mapped to the same region (eg. Su(var)306, 328 and 340). In addition, from both second and third chromosome suppressor mutations, some were homozygous viable mutations, again two suppressors inthe same genome. Visual examination showed that the eyes of homozygous flies generally have more pigment than the eyes of heterozygous flies.

From unstable second chromosome suppressor mutations, four enhancer mutations (En(var)KD201, 202, 203 and 204) were derived. Since these four enhancer mutations can enhance both w^{m4} and B^{sv} variegating alleles, they are probably general modifiers of position effect variegation. Unlike the suppressor mutations from which they were derived, these enhancer mutations are lethal as homozygotes. Complementation tests demonstrated that these four enhancer

mutations belong to the same lethal complementation group. Genetic mapping put one of the enhancer mutations, En(var)KD201, at 17 ± 1.0 on 2L of the second chromosome between *dp* (24E2-25A2) and *b* (34E2-35D1). In this region, previous studies identified a cluster of suppressor and enhancer mutations (Reuter and Szidonya, 1983; Sinclair *et al.*, 1983; Locke *et al.*, 1988).

How were the enhancer mutations described herein derived from suppressor mutations stocks? What is the relationship, if any, between the suppressor and the enhancer mutations? The phenotypic interaction between all four pairs showed that the pigment values of En(var)/Su(var) flies were between that of the Su(var)/CyO and En(var)/CyO, indicating that the interaction is additive but not epistatic. Since Southern blotting showed that there is no P-element present in the genome of any second chromosome suppressor mutations, the mutations may not be caused by the insertion of P-element. It is possible that the original suppressor mutations were caused by the insertion of some non-P-element transposable element. If these elements became unstable, they could move, thereby reverting the suppressor mutation, and insert into a classII modifier gene locus, thereby inducing an enhancer mutation. But this possibility appears unlikely, since it proposes that the transposable element excises precisely and insert into the same classII modifier gene in each of four cases. Another possibility is that the suppressor and the enhancer mutations affect the same class II gene(s) in an apparent dosage dependent manner. The original suppressor mutation is caused by the insertion of a transposable element, which results in elevated expression of that gene possibly

by blocking a negative regulatory elements. When this transposable element moves out, a deletion of that gene was also created, which result in an enhancer mutation.

As previous described, most modifier genes act in a dosage-dependent manner (Locke *et al.*, 1988; Reuter *et al.*, 1990). Some genes when duplicated act as enhancer, but when deleted or mutated act as suppressor. On the other hand, some modifier genes have a suppressor effect if they are duplicted but have an enhancer effect when deleted or mutated (Locke *et al.*, 1988).

The cross scheme used in my experiment was designed to screen for dominant suppressor mutations, consequently no recessive mutations were recovered. No recessive mutations of PEV modifier genes have been reported. This is probably due to the ease of recovering dominant suppressor and enhancer mutations since many of modifier genes are dosage dependent (Locke *et al.*, 1988). Therefore, simple loss-of-function mutations or duplication will act as dominant modifiers. Additionally, most of modifier mutations are recessive lethal. It is impossible to recover these recessive mutations. But there is another possibility. since so many modifier dominant mutations have already described, very few people pay attention to isolating recessive mutations.

2. Parental Source Effect of w^{m4}

The contribution of variegating alleles by different parents can affect the variegation phenotype of the progeny. These have been called the parental source effect (for review see Spofford, 1976). For example $Dp(1;3)N^{264\text{-}58a}$ is a w^+ variegating allele, in which a 20-

band segment of X chromosome containing w^+ is relocated to the proximal heterochromatin of 3L (Sutton, 1940). Two different stocks, designated as Dpf and Dp^a, were later established from $Dp(1;3)N^{264\text{-}58a}$. These two $Dp(1;3)N^{264\text{-}58a}$ stocks differed in their effects on the variegated expression of the $white^+$ gene. In the same genetic background, Dpf flies have more pigment than Dp^a flies. Spofford (1961) and Hessler (1961) found that when Dpf stock was used, a more extreme variegated phenotype could only be transmitted to the progeny through the egg rather than through the sperm. In contrast, the rearrangement from Dp^a produced a more extreme variegation when it was transmitted through the sperm than through the egg.

In this work, for the first time, it was shown that the parental source of the w^{m4} chromosome can affect the expression of w^{m4} phenotype in the progeny. Eye pigment studies showed that three w^{m4} variegation stocks from our lab have different amounts of eye pigment in males and females. All three sets of reciprocal crosses among these stocks showed that the progeny with the same genotype from a reciprocal cross expressed a different phenotype forw^{m4} vareigation. Both male and female progeny from one side of a reciprocal cross contained significantly more pigment than the other cross.

At this time, we still not know whether these parental effects are maternal, paternal or some combination of both. It is very difficult to design experiments that will distinguish between maternal and paternal effects since the final phenotype of w^{m4} variegation can be affected by many genetic and environmental

factors, such as, developmental timing, rearing-temperature, modifier genes, Y-chromosomes, in addition to the parental source of the variegating allele. It is extremely difficult to design an experiment to study these effects separately.

Two mechanisms can be proposed to account for the observed parental effects: (1). Some maternally expressed gene products can affect the heterochromatinization of the variegating regions containing the w^+ gene. These genes are expressed during oogenesis, and therefore their effects can only be transferred to the progeny *via* the eggs. This mechanism can only explain the maternal effects. (2). Both maternal and paternal effects can be explained by the following hypotheses. The genetic background can affect the extent of heterochromatin spreading toward the regions having w^+ genes. The heterochromatization in males and females is generally different since the genetic background in males is different from females (XY vs XX). Moreover, the state of heterochromatinization in males and females might be "imprinted" during the formation of sperm and egg, and then transmitted (inherited) directly to their progeny. This differential heritable chromatin configuration (heterochromatinization in this case) could be imposed by differential DNA methylation of sperm and egg genome or differential expression of some chromosomal proteins (such as some heterochromatin specific-binding proteins) in sperm and egg (for review see Monk, 1987). However, there is no detectable methylation in Drosophila DNA (Vrieli-Shoval *et al.*, 1982; Achwal *et al.*, 1984) and therefore it is more likely that any parental effect is due to differential expression of some heterochromatin specific-

binding proteins in the sperm and/or egg. Even though the progeny from a reciprocal cross have the same genotype, they may show a different w^{m4} variegation phenotype since the heterochromatinization of the *white*$^{+}$ gene inherited from each parent could be different.

3. Y-chromosome effects on the w^{m4} variegation

It is well known that an extra Y-chromosome can suppress position effect variegation while loss of a Y-chromosome will enhance variegation (Gowen and Gay, 1934). Early studies suggested that different Y-chromosome regions had different effects on position effect variegation and there exist some suppressor regions on the Y-chromosome (Baker and Spofford, 1959; Brosseau, 1964). More recently, Dimitri and Pisano (1989) found that the suppression effect of a Y-chromosome on position effect variegation appears to depend only on the amount of the Y-chromosome present in the genome and is not due to any discrete Y-chromosome regions. This suggestion is consistent with the view that the effect of a Y-chromosome on variegation acts via a source-sink effect where proteins are titrated away from the site of a variegating gene by heterochromatin on the Y-chromosome in somatic tissues. In the present work, I have shown that different Y-chromosomes have different effects on the expression of variegation of w^{m4}. The Y-chromosome in stock Su(var)KD328(A) can dramatically decrease the eye pigment of a very strong suppressor mutation Su(var)KD328 in the males to a level comparable with typical w^{m4}. On the other hand, the Y-chromosome in stock Su(var)KD328(B) alone mutated

from Su(var)KD328(A) can restore the suppressor effect of Su(var)KD328 in the males. What is the difference between these? What changes occurred in the Y-chromosome of Su(var)KD328(B) to make it different from the Y-chromosome of Su(var)KD328(A)? Based on a recent view (Dimitri and Pisano, 1989) that the suppression effect of a Y-chromosome is dependent on the amount of heterochromatin present in the genome, it is possible that the Y-chromosome of Su(var)KD328(A) could have a deletion of Y-heterochromatin. Therefore it would enhance variegation w^{m4}. It is possible that a Y-chromosome region in the Su(var)KD328(A) line has been duplicated, to give the Y-chromosome of the Su(var)KD328(B) line. This duplicated Y-chromosome region in Su(var)KD328(B) could permit the suppressor mutation in the Su(var)KD328 (B) line to suppress variegation w^{m4}.

4. Problems in Cloning Modifier Genes of PEV

It has been estimated that there are about 30-100 PEV modifier loci in the genome of *Drosophila melanogaster* (Locke *et al.*, 1988; Wustmann *et al.*, 1989). Cloning of these genes is a prerequisite for studying their structure and function at the molecular level. However, only two modifier genes of PEV have been reported cloned and studied at the molecular level (Eissenberg *et al.*, 1990; Reuter *et al.*, 1990).

One gene, Suvar(3)7, was cloned from a large chromosomal walk (Reuter *et al.*, 1990). Reuter *et al.* (1987) mapped the Suvar(3)7 gene to a previously cloned region; 87DE of the third chromosome. Furthermore this gene was physically mapped to a

10.4 kb DNA fragment by deletion and *in situ* hybridization analyses (Reuter *et al.*, 1987). By inserting different pieces of this 10.4 kb genomic DNA into a P-element transposon and microinjecting each construct into early embryos, they found that a 6.5 kb *Eco*R V fragment could rescue the function of the Suvar(3)7 mutation. Then they isolated a cDNA clone of this gene by using this DNA fragment as a probe. The sequence of this cDNA clone revealed an encoded 932-amino acid protein having five potential Zinc-fingers of the Cys-His_2 type.

Another suppressor gene, Su(var)205, was also cloned by an indirect method. When fluorescence-labeled monoclonal antibodues prepared against fractionated nuclear proteins of *D. melanogaster* embryos were used to stain the polytene chromosome of third-instar larvae, James and Elgin (1986) identified a heterochromatin-specific chromosomal protein called HP-1. The cDNA encoding this protein was cloned by screening a λgt11 cDNA expression library using fluorescence-labeled antibodies as a probe. By *in situ* hybridization, this gene was localized to 29A on the polytene chromosome map. In this region Sinclair *et al.* (1983) had previously mapped a suppressor mutant Su(var)205. The cDNA of Su(var)205 was cloned by using HP-1 cDNA as probe. The comparison of the cDNA sequence between the HP-1 gene and the Su(var)205 gene suggested that the Su(var)205 mutation is a point mutation in a heterochromatin-specific nonhistone chromosomal protein HP-1 gene causing aberrant splicing of the HP-1 gene transcript (Eissenberg *et al.*, 1990).

Since the discovery of the molecular basis of P-M hybrid dysgenesis, P-element tagging has become a very powerful

technique to clone genes from the Drosophila genome, and many genes have been cloned by P-element tagging (Pirrotta, 1986). Since there are about 20-50 PEV modifier genes present in the genome of *Drosophila melanogaster* (Locke *et al.*, 1988), why have not any of these genes been cloned by P-element tagging?

Locke *et al.* (1988) isolated 12 enhancer mutations of position effect variegation by P-element mutagenesis with the initial purpose of cloning some enhancer or suppressor genes (Tartof *et al.*, 1989). Unexpectedly, these mutations were not due to the simple insertion of P-elements. Instead, 10 of these mutations were cytologically visible duplications or deficiencies. From these experiments, they proposed a model suggesting how the heterochromatic proteins coordinately assemble to form heterochromatin. But no modifier genes were cloned by P-element tagging.

My original purpose of screening for suppressor mutations by pUChsneo and transposase producing Δ2-3 P-elements mutagenesis was to clone these PEV modifier genes by plasmid rescue (Perucho *et al.*, 1980). However, the results of Southern blotting analyses were unexpected. Except for the discovery that there was an intact pUChsneo P-element sequence in the genome of Su(var)KD340, there is no intact pUC8 sequence present in the genome of the other suppressor mutations. Moreover, there are no P-element sequences present even in the genome of any second chromosome suppressor mutants. How were these non-P-element mutations produced? Several possible mechanisms can be suggested. 1). It is possible that these mutations were induced by P-element insertion, but later the P-element jump out and the excision was not precise. Therefore, the

mutation was created but no P-elements remained in the genome of these mutations. 2). These mutations are possibly induced by other transposable elements existing in the genome of Drosophila. such as Copia-like elements, Foldback element and I-R elements (see introduction for more information). 3). These mutations are probably pre-existed in the parental stocks. In this screening process, these mutations were recovered. In many cases, cluster mutations were isolated from the same bottle. This mutations are very likely to be derivred from the pre-existed mutations in the parental stocks.

It has been pointed out that the number of PEV modifier genes detected by mutagenesis (EMS, X-rays or P-elements) is lower than the number detected by deficiency mapping. It is very likely that for some loci only amorphic or strongly hypomorphic mutations might result in a detectable dominant suppressor or enhancer phenotype. Other loci might actually be repeated genes which can only be detected by deficiencies (Reuter *et al.*, 1987). Thus for some modifier genes the insertion of a P-element may not induce a visible modifier phenotype. This may be the reason for the difficulty in cloning modifier genes by P-element tagging.

5. Further work to clone Su(var)KD340 gene

Southern analyses indicated that there is an intact pUChsneo P-element sequence present in the genome of Su(var)KD340. To date, it is not clear whether this suppressor mutation is caused by the insertion of the pUchsneo P-element or not. To confirm whether the Su(var)KD340 was caused by the insertion of the pUChsneo P-

element, more experiments have to be done: 1). *In situ* hybridization. The Su(var)KD340 has been mapped to 55.6 ± 1.0 location on the third chromosome corresponding to the polytene chromosome region 88A-90A. If the mutation was caused by the insertion of a pUChsneo P-element, labelled probes using either pUC19N or P-element sequences should hybridize to this region. 2). Studying the revertants of Su(var)KD340. An advantage of using P-element tagging to clone genes in *Drosophila melanogaster* is that revertants of the original mutations can be obtained easily by genetic crosses. To obtain revertants of Su(var)KD340, crosses can be made between Su(var)KD340 and the stocks having a Δ2-3 transposase-producing P-element. The inserted pUChsneo P-element can then be mobilized by the transposase produced by the Δ2-3 transposon and excised from the insertion site. If the excision is precise, then the suppressor gene function should be restored. The revertants of suppressor mutations can be selected in the subsequent progeny. By screening 20,000 flies, one revertant of Su(var)KD340 has already been obtained. Visual examination showed that both male and female eyes contained very little pigment; just like the w^{m4} phenotype. By screening more flies, more revertants could be recovered. Once several revertants are isolated, the Southern analyses using pUC19N and P-element sequences as probes can be carried out. Compared to Su(var)KD340, the change or loss of pUChsneo P-element sequences in the revertants would support that idea that the original mutation was caused by the insertion of a pUChsneo P-element.

If it can be confirmed that Su(var)KD340 phenotype is caused by the insertion of the pUChsneo P-element, a plasmid rescue experiment to clone the flanking sequence of the Su(var)KD340 gene can then be carried out (Perucho *et al.*, 1980; Kidwell, 1986). The genomic DNA of Su(var)KD340 could be digested with *Eco*RI, *Bam*HI or *Sal*I, then diluted under conditions that allow circularization of the plasmid fragment, and ligated. The ligated mixture will then be used to transform high-efficiency competent cells. Plasmid bearing cells will be selected in media containing ampicillin. In this manner clones containing the pUC8 and flanking sequences of Su(var)KD340 gene can be recovered. The flanking sequence could then be analyzed and isolated by restriction enzyme digestion and later serve as probes for screening a genomic library of wild type DNA. As a matter of fact, a 2 kb DNA fragment has been rescued from the *Sal*I-*Xho*I double digestion of *P[pUChsneo](9C); mwh red e* (#26), which is the stock for providing pUChsneo P-element for mutagenesis (see Figure 1). So if the Su(var)KD340 phenotype could be confirmed to be caused by the insertion of a pUChsneo P-element, it should not be difficult to clone the gene by plasmid rescue.

V. References

Achwal, C. W.; Ganguly, P. and Chandra, H. S. 1984. Estimation of the amount of 5-methlcytosine in *Drosophila melamogaster* DNA by amplified ELISA and photoacoustic spectroscopy. EMBO J. 3, 263-266.

Ananiew, E. V. and Gvozdev, U. A. 1974. Changed pattern of transcription in polytene chromosomes of *Drosophila melanogaster* resulting from eu-heterochromatin rearrangement. Chromosoma 45, 173-191.

Arrighi, F. E. and Hsu, T. C. 1971. Localization of heterochromatin in human chromosome. Cytogenetics 10, 81-86.

Ashburner, M. 1989a. Mutation and mutagenesis In Drosophila: a laboratory handbook. Cold Spring Harbor Laboratory Press. pp 299-418.

Ashburner, M. 1989b. Hybrid dysgenesis and related phenomena In Drosophila: a laboratory handbook. Cold Spring Harbor Laboratory Press. pp 969-1015.

Baker, W. K. 1953. V-type position effects of a gene in *Drosophila virilis* normally located in heterochromatin. Genetics 38, 328-344.

Baker, W. K. 1968. Position effect variegation. Adv. Genet. 14, 133-169.

Baker, W. K. and Spofford, J. B. 1959. Heterochromatic control of position effect variegation in *Drosophila*. Univ. Texas Publ. 5914, 135-154.

Becker, H. J. 1961. Untersuchungen zur wirkung des heterochromatin auf die genmanifestierung bei *Drosophila melanogaster*. Verhandl. Deut. Zool. Ges. Bonn-Rhein Suppl. 24 pp. 283-291.

Becker, H. J. 1966. Genetic and variegation mosaics in the eye of Drosophila. In Current Topics in Developmental Biology (A. A. Moscona and A. Monroy, eds), Vol. 1 pp 155-171, Academic press, New York.

Berlowitz, L. 1965. Correlation of genetic activity, heterochromatinization, and RNA metabolism. Proc. Nat. Acad. Sci. 53, 68-73.

Bernard, J. 1988. The biology of heterochromatin in Heterochromatin: Molecular and structural aspects Edited by Rams, V. Cambridge University press. 1-147.

Bridges, C. B. 1936. The Bar "gene" a duplication. Science 83, 210-211.

Brosseau, G. E., JR. 1960. V-type effects influencing the action of *Bar* locus in *Drosophila*. Genetics 45, 979.

Brosseau, G. E., JR. 1964. Evidence that heterochromatin does not suppress V-type position effect. Genetics 50, 237.

Brown, S. W. 1966. Heterochromatin. Science 151, 417-425.

Candido, E. P. M.; Reeves, R. and Davie, J. R. 1978. Sodium butyrate inhibits histone deacetylation in cultured cells. Cell 14, 105-113.

Charlesworth, B. 1988. Driving genes and chromosomes in *Drosophila melanogaster*. Genetics 86, 321-355.

Cooley, L.; Kelley, R. and Spradling, A. 1988. Insertion mutagenesis of the Drosophila genome with single P-elements. Science 239, 1121-1128.

Cowell, J. K. and Hartmann-Goldstein, I. 1980. Contrasting response of euchromatin and heterochromatin to translocation in polytene chromosome of *Drosophila melanogaster*. Chromosoma 79, 329-340.

Demerec, M. 1941. The nature of changes in the *white-Notch* region of the X chromosome of *Drosophila melanogaster*. Proc. 7th Intl. Congr. Genet. pp 99-103.

Demerec, M. and Slizynska, H. 1937. Mottled *white* 258-18 of *Drosophila melanogaster*. Genetics 22, 641-649.

Devlin, R. H.; Bingham, B. and Wakimoto, B. T. 1990. The organization and expression of the light gene of *Drosophila melanogaster*. Genetics 125, 129-140.

Dimitri, P. and Pisano, C. 1989. Position effect variegation in *Drosophila melanogaster*: relationship between suppression effect and the amount of Y-chromosome. Genetics 122, 793-800.

Dobzhansky, T. H. 1936. Position effects on Genes. Biol. Rev. 11, 364-384.

Dorn, R.; Heymann, S.; Lindingkeit, R. and Reuter, G. 1986. Suppressor mutation of position-effect variegation in *Drosophila melanogaster* affecting chromatin properties. Chromosoma (Berl) 93, 389-403.

Dubinin, N. P. and Sidorov, B. N. 1934. Relation between the effect of a gene and its position in the system. Am. Nat. 68, 377-381.

Dubinin, N. P. and Sidorov, B. N. 1935. The position effect of the hairy gene. Biol. Zh. Mosk. 4, 555-568.

Eissenberg, J. C. 1989. Position effect variegation in Drosophila: Towards a genetics of chromatin assembly. Bioessays 11, 14-17.

Eissenberg, J. C.; Cartwright, I. L.; Thomas, G. H. and Elgin, S. C. R. 1985. Selected topics in chromatin structure. Ann. Rev. Genet. 19, 485-536.

Eissenberg, J. C.; Elgin, S. C. R and James, T. C. 1987. A heterochromatin specific chromosomal protein in *Drosophila melanogaster*. Genetics 116, s4.

Eissenberg, J. C.; James, T. C.; Foster-Hartnett, D. M.; Hartnett, T.; Ngan, V. and Elgin, S. C. R. 1990. Mutation in a heterochromatin-specific chromosomal protein is associated with suppressor of position-effect variegation in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA. 87, 9923-9927.

Elgin, S. C. R. 1975. Chromosomal proteins and chromatin structure. Ann. Rev. Biochem. 44, 725-74.

Ephrussi, B. and Herold, J. L. 1944. Studies of eye pigments of Drosophila. I. Methods of extraction and quantitative estimation of the pigmant components. Genetics 29, 148-175.

Felsenfeld, G. and McGhee, J. D. 1986. Structure of the 30 nm chromatin fiber. Cell 44, 375-377.

Finch, J. T. and Klug, A. 1976. Solenoidal model for superstructure in chromatin. Proc. Natl. Acad. Sci. USA 73, 1897-1901.

Ganetzky, B. 1977. On the components of segregation distortion in *Drosophila melanogaster*. Genetics 86, 321-355.

Goodnight, J. H. and Stanish, W. M. 1990. The T TEST Procedure in SAS/STAT User's Guide Release 6.03 Edited by David, M. D. Chapter 33, pp941-945.

Gowen, J. W. and Gay, E. H. 1934. Chromosome constitution and behavior in ever-sporting and mottling in *Drosophila melanogaster*. Genetics 19, 189-208.

Grigliatti, T. 1986. Mutagenesis. In Drosophila: A Practical Approach, Edited by D. B. Roberts. IRL Press, Oxford. pp 39-28.

Hannah, A. 1951. Localization and function of heterochromatin in *Drosophila melanogaster*. In Advances in Genetics Edited by Demerec, M. Academic Press New York, Vol IV, 87-125.

Hartmann-Goldstein, I. J. 1976. On the relationship between heterochromatinization and variegation in Drosophila, with special reference to temperature-sensitive periods. Genet. Res. 10, 143-159.

Heitz, E. 1928. Das heterochromatin der moose. I. Jahrb. Wissensch Bot. 69, 726-818.

Heitz, E. 1933. Die somatische heteropknose bei *Drosophila melanogaster* und ihre genetische bedeutung. Z. Zellforsch. 20, 237-287.

Heitz, E. 1934. Uber α and β heterochromatin sowie konstanz und bau der chromomeren loci. Drosophila Biol. Zbl. 54, 588-609.

Henikoff, S. 1979. Position effects and variegation enhancers in an autosomal region of *Drosophila melanogaster*. Genetics 93, 105-115.

Henikoff, S. 1981. Position-effect variegation and chromosome structure of a heat shock puff in Drosophila. Chromosoma 83, 381-393.

Henikoff, S. 1990. Position effect variegation after 60 years. TIG Vol. 16, No.12, 422-426

Henikoff, S. and Dreesen, T. D. 1989. Trans-inactivation of *Drosophila* brown gene: Evidence for transcriptional repression and somatic pairing dependence. Proc. Natl. Acad. Sci. USA 86, 6704-6708.

Hessler, A. Y. 1958. V-type position effects at the light locus in *Drosophila melanogaster.* Genetics 43, 395-403.

Hessler, A. Y. 1961. A study of parental modification of variegated position effects. Genetics, 463-484.

Hilliker, A. J. 1976. Genetic analysis of the centromeric heterochromatin of chromosome 2 of *Drosophila melanogaster*: deficiency mapping of EMS-induced lethal complementation groups. Genetics 83, 765-782.

Hilliker, A. J. and Appels, R. 1980. The genetic analysis of *Drosophila melanogaster* heterochromatin. Cell 21, 607-619.

Hinton, T. and Goodsmiths, W. 1950. An analysis of phenotypic reversions at the brown locus in Drosophila. J. Exp. Zool. 114, 103-114.

Ish-Horowicz, D. and Pinchin, M. 1979. Genetic and molecular analysis of the 87A7 and 87C1 heat-inducible loci of *D. melanogaster.* Cell 18, 1351-1358.

James, T. C. and Elgin, S. C. R. 1986. Identification of a nonhistone chromomal protein associated with heterochromatin in *Drosophila melanogaster* and its gene. Mol. Cell Biol. 6, 3862-3872.

Judd, B. H. 1955. Direct proof of a variegated-type position effect at the white locus in *Drosophila melanogaster.* Genetics 40, 739-740.

Kaufmann, B. P. 1942. Reversion from roughest to wild-type in *Drosophila melanogaster.* Genetics 27, 537-549.

Kaufmann, B. P. 1946. Organization of the chromosome I, Break distribution and chromosome recombination in *Drosophila melanogaster*. J. Exp. Zool. 102, 293-320.

Kedes, L. H. 1979. Histone genes and histone messenges. Annv. Rev. Biochem. 48, 837-870.

Kellum, R. and Schedl, D. 1991. A position effect assay for boundaries of higher order chromosomal domains. Cell 64, 941-950.

Kidwell, M. G. 1986. P-M mutagenesis In Drosophila: A Practical Approach, Edited by D. B. Roberts. IRL Press, Oxford. pp 59-82.

Kornber, J. S. and Kauffman, S. A. 1986. Variegated expression of the *Sgs-4* locus in *Drosophila melanogaster*. Chromosoma 94, 205-216.

Laski, F. A.; Rio, D.C. and Rubin, G. M. 1986. Tissue specificity of Drosophila P-element transposition is regulated at the level of mRNA splicing. Cell 44, 7-19.

Lefever, G., JR. 1976. A photographic representation and interpretation of the polytene chromosomes of *Drosophila melanogaster* salivary glands. pp. 31-66. In: The Genetics and Biology of *Drosophila*, Vol. 1a Edited by Ashburner, M. and Novitskl, E. Academic Press, New York.

Levinger, L. and Varshavsky, A. 1982. Protein D1 preferentially binds to A+T-rich DNA *in Vitro* and is a component of *Drosophila melanogaster* nucleosomes containing A+T-rich satellite DNA. Proc. Natl. Acad. Sci. USA 79, 7152-7156.

Lewis, E. B. 1950. The phenomenon of position effect. Adv. Genet. 3, 73-115.

Lifton, R. P.; Goldberg, M. L.; Karp, R. W. and Hogness, D. S. 1978. The organization of the histone genes in Drosophila melanogaster: functional and evolutional implications. Cold Spring Harbor Symp. Quant. Biol. 42, 1047-1051.

Lima-de-Faria, A.; Nilsson, B.; Cave, D.; Puga, A. and Jaworska, H. 1968. Tritium labelling and cytochemistry of extra DNA in Acheta. Chromosoma 25; 1-20.

Lindsley, D. L. and Grell, E. H. 1968. Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

Locke, J,; Kotarski, M. A. and Tartof, K. D. 1988. Dosage-dependent modifiers of position-effect variegation in Drosophila and a mass action model that explains their effects. Genetics 120, 181-198.

Maniatis, T.; Fritsch, E. and Sambrook, J. 1982. Molecular cloning: A Laboratory Manual (Cold Spring Harbor Lab., Cold Spring Harbor, N Y).

Manuelidis, L. 1982. Repeated DNA sequences and nuclear structures. In Genome Evolution Edited by Dover, G. A. and Flavell, R. B. London, Academic Press. pp 263-283.

McGhee, J. D. and Felsenfeld, G. 1980. Nucleosome structure. Ann. Rev. Biochem. 49, 1115-1156.

Michailidis, J.; Murray, N. D. and Graves, J. A. M. 1988. A correlation between development time and variegated position effect in *Drosophila melanogaster*. Genet. Res. Camb. 52, 119-123.

Monk, M. 1987. Genomic imprinting. Genes and development 2, 921-925.

Moore, G. D.; Procunier, J. D.; Cross, D. P. and Grigliatt, T. A. 1979. Histone gene deficiencies and position-effect variegation in Drosophila. Nature 282-314.

Moore, G. D.; Sinclair, D. A. and Grigliatt, T. A. 1983. Histone gene multiplicity and position-effect variegation in *Drosophila melanogaster*. Genetics 105, 327-344.

Mottus, R.; Reeves, R. and Grigliatt, T. A. 1980. Butyrate suppression of position-effect variegation in *Drosophila melanogaster*. Mol. Gen. Genet. 178, 465-469.

Muller, H. J. 1930. Types of visible variations induced by X-rays in Drosophila. J. Genet. 22, 299-334.

Muller, H. J. and Painter, T. S. 1932. The differentiation of the sex chromosomes of Drosophila into genetically active and inert regions. Z. Abstam. Vererbungsl. 62, 316-365.

Novitski, E. 1961. The regular reinversion of the roughest inversion. Genetics 46, 111-117.

Panshin, I. B. 1935. New evidences for the position effect hypothesis, C. R. (Doklady) Acad. Sci. U.R.R.S. 9(4), 85-88.

Pardue, M. L.; Kedes, L. H.; Weinberg, E. S. and Birnstiel, M. L. 1977. Localization of sequences coding for histone messenger RNA in the chromosomes of *Drosophila melanogaster*. Chromosoma (Berl) 63, 135-151.

Paulson, J. R. and Laemmli, U. K. 1977. The structure of histone-depleted metaphase chromosomes. Cell 12, 817-828.

Peacock, W. J.; Appels, R.; Dunsmuir, P.; Lobe, A. R. and Gerlach, W. L. 1977. Highly repeated DNA sequences: chromosomal localization and evolutionary conservation. In International Cell Biology 1976-1977 Edited by Brinkley, B. R. and Porter, K. R. The Rockefeller University Press, New York, 494-506.

Perucho, M., Hanahan, D. and Wiglar, M. 1980. Genetic and physical linkage of exogenous sequences in transformed cells. Cell 22, 309-317.

Pimpinelli, S. and Dimitri, P. 1989. Cytogenetic analysis of segregation distortion in *Drosophila melanogaster*: The cytological organization of the Responder (Rsp) locus. Genetics 121, 705-772.

Pimpinelli, S.; Sullivan, W.; Prout, M. and Sandler, L. 1985. On biological functions mapping to the heterochromatin of *Drosophila melanogaster*. Genetics 109, 701-724.

Pirrotta, V. 1986. Cloning Drosophila genes In Drosophila: A Practical Approach, Edited by D. B. Roberts. IRL Press, Oxford. pp 83-110.

Reuter, G. and Szidonya, J. 1983. Cytogenetic analysis of variegation suppressors and a dominant temperature-sensitive lethal in region 23-26 of chromosome 2L in *Drosophila melanogaster*. Chromosoma 88, 277-285.

Reuter, G. and Wolff, I. 1981. Isolation of dominant suppressor mutations for position effect variegation in *Drosophila melanogaster*. Mol. Gen. Genet. 182, 516-519.

Reuter, G.; Dokn, R.; Wustmann, G.; Friede, B. and Rauh, G. 1986. Third chromosome suppressor of position-effect variegation loci in *Drosophila melanogaster*. Mol. Gen. Genet. 202, 481-487.

Reuter, G.; Gausz, J.; Gyurkovics, H.; Friede, B.; Bang, R.; Spierer, A.; Hall, L. M. C. and Spierer, P. 1987. Modifiers of position-effect variegation in the region from 86C to 88B of the *Drosophila melanogaster* third chromosome. Mol. Gen. Genet. 210. 429-436.

Reuter, G.; Giarre, M.; Farah, J.; Gausz, J.; Spierer, A. and Spierer, P. 1990. Dependence of position-effect variegation in Drosophila on dose of a gene encoding an unusual Zinc-finger protein. Nature 344, 219-233.

Reuter, G.; Werner, W. and Hoffman, H. J. 1982. Mutants affecting position-effect heterochromatinization in *Drosophila melanogaster*. Chromosoma 85, 539-551.

Robertson, H. M.; Preston, C. R.; Phillis, R. W.; Johnson-Schlitz. D. M.; Ben, W. K. and Engels, W. R. 1988. A stable genomic source of P-element transposase in *Drosophila melanogaster*. Genetics 118, 461-470.

Rushlow, C. A. and Chovnick, A. 1984. Heterochromatin position-effect at the rosy locus of *Drosophila melanogaster*: Cytological, genetic and biochemical characterization. Genetics 108, 589-602.

Rushlow, C. A.; Bender, W. and Chovnick, A. 1984. Studies on the mechanism of heterochromatic position effect at the rosy locus of *Drosophila melanogaster*. Genetics 108, 603-615.

Sandler, I. and Golic, K. 1985. Segregation distortion in Drosophila. TIG. 181-185.

Schalet, A. 1969. Three Y-suppressed phenotypes associated with X chromosomes carrying *In(1)* ^{ml}, Dros. Inf. Serv. 44, 87.

Schultz, J. 1936. Variegation in Drosophila and the inert chromosome regions. Proc. Nat. Acad. Sci. USA. 22, 27-33.

Schultz, J. 1936. Variegation in Drosophila and the inert chromosome regions. Proc. Natl. Acad. Sci. USA. 22, 27-33.

Schultz, J. 1941. The function of heterochromatin. Proc. 7th Inter. Congr. Genet Edinbergh, pp 257-262.

Schultz, J. 1950. Interrelation of factors affecting heterochromatin-induced variegation in Drosophila. I. White variegation. Genetics 57, 751-766.

Schupbach, T. and Wieschaus, E. 1988. Female sterile mutations on the second chromosome of *Drosophila melanogaster*. I. Maternal effect mutations. Genetics 121, 101-117.

Sinclair, D. A. R.; Mottus, R. C. and Grigliatti, T. A. 1983. Genes which suppress position-effect variegation in *Drosophila melanogaster* are clustered. Mol. Gen. Genet. 191, 326-333.

Sinclair, D.A.R.; Lloyd, V. K. and Grigliatti, Y.A. 1989 Characterization of mutations that enhance position-effect variegation in *Drosophila melanogaster*. Mol. Gen. Genet. 216, 328-333.

Skinner, L. G. and Ockey, C. H. 1971. Isolation, fractionation and biochemical analysis of the metaphase chromosomes of *Microtus agrestis*. Chromosoma 35, 125-142.

Southern, E. M. 1975. Detection of specific sequences among DNA fragments separeated by gel electrophoresis. J. Mol. Biol. 98, 503-517.

Spofford, J. B. (1967). Single-locus modification of position effect variegation in *Drosophila melanogaster*. I. white variegation. Genetics 57, 751-766.

Spofford, J. B. 1961. Parental control of position effect variegation. II. Effect of sex of parent in contributing white-mottled rearrangement in *Drosophila melanogaster*. Genetics 46, 1151-1167.

Spofford, J. B. 1976. Position effect variegation in Drosophila. In The Genetics and Biology of Drosophila Vol. 1c, 955-1018.

Spradling, A. C. and Rubin, G. M. 1982. Transposition of cloned P elements into *Drosophila* germ line chromosome. Science 218, 341-347.

Steller, H. and Pirrotta, V. 1985. A transposable P-element vector that confers selectable G418 resistance to Drosophila larval. EMBO J. 4, 167.

Stern, C. 1960. Dosage composation-development of a concept and new facts. Can. J. Genet. Cytol. 2, 105.

Strickberger, M. W. 1959. An improved salivary technique. *Drosophila* inform. Serv. 33, 183.

Stubblefield, E. and Wray, W. 1971, Architecture of the chinese hamster metaphase chromosome. Chromosoma 32, 262-294.

Sturtevant, A. H. 1925. The effects of unequal crossing-over at the *Bar* locus of Drosophila. Genetics 16, 267-90.

Sueoka, N. 1961. Varieation and heterogeneity of base composition of deoxyribonucleic acids: A compilation of old and new data. J, Mol. Biol. 3, 31-40.

Sutton, E. 1940. The structure of salivary gland chromosomes of *Drosophila melanogaster* in exchanges between euchromatin and heterochromatin. Genetics 25, 534-540.

Sved, J. A. 1976. Hybrid dysgenesis in *Drosophila melanogaster*: A possible explanation in term of spatial organization of chromosomes. Aust. J. Biol. Sci. 29, 375-388.

Tartof, K. D. and Hobbs, C. A. 1988. New cloning vectors and techniques for easy and rapid restriction mapping. Gene 67, 169-182.

Tartof, K. D.; Bishop, C.; Jones, M.; Hobbs, C. A. and Locke, J. 1989. Towards an understanding of position effect variegation. Developmental Genetics 10, 62-176.

Tartof, K. D.; Hobbs, C. and Jones, M. 1984. A structural basis for variegating position effects. Cell 37, 869-878.

Urieli-Shoval, S.; Gruenbaum, J, S. and Razin, A. 1982. The absence of detectable methylated bases in *Drosophila melenogaster* DNA. FEBS lett. 146, 148-152.

Van Holde, K. E. 1989. Chromatin. Springer-Verlag, New York Inc.

Vdvardy, A.; Maine, E. and Schedl, P. 1985. The 87A7 chromosome identification of novel chromatin structures flanking the heat shock locus that may define the boundaries of higher order chromosomal domains. Cell 64, 941-950.

Wargent, J. M.; Hartmann-Goldstein, I. and Goldstein, D. J. 1974. Relationship between DNA content of polytene chromosome bands and heterochromatinzation in Drosophila. Nature 248, 55-57.

Wargent, J.M. 1971. Position-effect variegation in wings of *Drosophila melanogaster*. Dros. Inf. Serv. 47, 91.

Wu, C. I.; Lyttle, T. W.; Wu, M. L. and Lin, G. F. 1988. Association between a sattlite DNA sequence and the Responder of Segregation Distorter in *D. melanogaster*. Cell 54, 179-189.

Wu, C. I.; True, J. R. and Johnson, N. 1989. Fitness reduction associated with the deletion of a satellite DNA array. Nature 341, 248-251.

Wustmann, G.; Szidonya, J.; Taubert, H. and Reuter, G. 1989. The genetics of position effect variegation modifying loci in *Drosophila melanogaster*. Mol. Gen. Genet. 217, 520-527.