Genetic and molecular analyses of suppressor of forked in Drosophila melanogaster

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Genetic and molecular analyses of suppressor of forked in Drosophila melanogaster

Fitch-Steensson, Cynthia Lynn, Ph.D.
Iowa State University, 1990
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Genetic and molecular analyses of suppressor of forked in Drosophila melanogaster

by

Cynthia Lynn Fitch-Steenson

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department: Genetics
Major: Molecular, Cellular, and Developmental Biology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa
1990
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Mutations at the suppressor of forked, su(f), locus are trans-acting, allele specific suppressors and enhancers of mutations at certain other loci. Su(f) is a trans-acting regulator whose normal product is necessary for proper transcription of these genes. The su(f) product regulates the transcription of transposable elements located within these genes. Our investigation includes a genetic analysis in which a number of new su(f) alleles were isolated, a developmental analysis in which the effects of new alleles and allelic combinations were characterized, and a molecular analysis in which a cloned genomic DNA fragment containing su(f) was used (in collaboration with Dr. K. O'Hare, Imperial College, London) to determine molecular lesions for the su(f) alleles. Genetic analyses of this locus included complementation studies of su(f) alleles and studies of suppression and enhancement at other target loci. These studies helped us to refine and confirm parts of our hypothesis which we call the Model of Action. The phenotypic effects of suppression and enhancement were proven to be genetically separable. Several new alleles of su(f) were isolated and characterized both molecularly and genetically. These alleles showed a Restriction Fragment Length Polymorphism when probed with the su(f) cloned fragments. These mutational lesions were incorporated into a modified molecular map of this region. Finally, these fragments were shown to hybridize to regions on the salivary gland chromosome that correlate to su(f). Our goal throughout this study was to gain a better understanding of trans-acting gene regulation during normal growth and development.
INTRODUCTION

General Introduction

An individual cell, in its small and simplistic appearance, contains all the instructions that direct its development and its very existence. Each cell contains a full set of chromosomes in which are found cryptically encoded instructions. These instructions are executed according to and depending upon particular DNA sequences called genes. The study of gene regulation and expression is of the utmost importance in the overall quest of understanding the mechanisms of heredity and development.

Many properties of eukaryotic gene regulation can be studied in single cell organisms, cell cultures, or in vitro environments. However, to better understand the overall intracellular, intercellular, and developmental results of gene regulation, an intact, in vivo, eukaryotic environment is an optimal tool (Rubin, 1988). The fruit fly, Drosophila melanogaster, serves as just such a tool. It is of small size and has a short life cycle which is a distinct advantage for the study of genetics, since large numbers of individuals for many generations can be screened quite easily. The study of this organism began with T.H. Morgan in 1909, and shortly thereafter, the observation of genetic mutations led to the establishment of a relationship between genes and chromosomes. Many genetic foundations have been laid since then and a great many are due to studies carried out using Drosophila (Rubin, 1988). Many advantages for genetic research exist using the experimental organism, Drosophila. Some of these are the
ease of chromosome manipulation and mutation as well as detailed phenotypic and physiological studies. This thesis contains a study of gene regulation and was carried out using *Drosophila* as an experimental organism primarily because of the rich history of the genetic mutations of suppressor loci and the interactions of the genes involved.

The control of gene expression is exerted through molecules that interact with DNA, with mRNA, and with gene products (proteins). These control molecules include enzymes and other proteins that help activate, enhance, or inhibit the transcription of particular genes. Perhaps the most common type of control is by a regulatory protein interacting directly with the transcription of a gene. Variations of this normal gene regulation may result in an over-abundance, a shortage of, or even an altered transcribed message. These variations may occur through many mechanisms such as modified binding to promoters or enhancers, to transposable elements, or even to encoded regulatory sequences, all resulting in a change in the rate and/or character of the transcribed message. This complicated interchange of genes and their products is the focus of this study; in particular, one gene, its product, and how it affects the regulation of other genes.

Several types of gene regulation are involved in a phenomena called suppression. "Suppression is defined as the reversal of a mutant phenotype due to a mutation at a site distinct from that giving rise to the mutant phenotype" (Kubli, 1986). This suppression of a mutant phenotype can be due to the interaction of alleles, (one type of intragenic suppression), or to the interaction of non-allelic mutant genes, (intergenic suppression). "Enhancement" is a functionally related term and is used to describe
mutations that increase the phenotype of other unlinked mutations (Parkhurst and Corces, 1985). The interaction of non-allelic mutant genes resulting in suppression can be at the transcriptional, translational, or post-translational level. This type of interaction between non-allelic mutant genes has also been termed trans-acting gene regulation. This type of regulation involves a product which must physically move to another location and perform its regulatory function. It is different from cis-acting regulation which implies the regulator is functioning on targets that are "in cis" that is, on the same chromosome. The term, "in cis" is used to indicate that mutant genes are on the same chromosome.

Suppression has been demonstrated in many organisms such as yeast, maize, and mice. In yeast, auxotrophic mutations his4-912 and his4-917 are caused by the insertion of a Ty element near the end of the coding region of HIS4 gene (Roeder and Fink, 1983). SPT (SPT= suppressor of Ty) genes can act as repressor loci. An spt3- null mutation can suppress the his4-917 mutation. This suppression occurs because the orientation of the Ty element is such that it is transcribed opposite to that of the HIS4 gene. However, spt3- does not suppress the his4-912 mutation. This is because the Ty element is orientated such that it is transcribed in the same direction as the HIS4 gene. In addition to the SPT3 suppressor gene, mutations at 6 other unlinked SPT genes lead to the suppression of HIS4. These mutations also display a wide variety of other mutations which affect mating, DNA repair, and growth (Winston et al., 1984a, and Winston et al., 1984b). Suppression in maize has been shown with several two-element controlling systems, such as AC-DS, (McClintock, 1950, and
McClintock, 1951) and Spm (Suppressor-mutator) (McClintock, 1965), which consist of unlinked genetic loci which have a variety of effects on insertion mutations. A recent example is shown in the photosynthetic mutant hcf*106 which is due to the insertion of a Mul element and is termed a Mu-suppressible mutation. When Mu is inactive, suppression of the mutant phenotype occurs. Homozygous hcf*106 plants containing predominantly modified Mul elements have a wild-type phenotype, while homozygous hcf*106 plants containing unmodified elements are mutant. These results were used to clone the hcf*106 locus and show that this suppression is mediated at the level of transcript accumulation (Martienssen et al., 1989). A mutation at the dilute (d) gene in mice, produces a pale coat color, and is due to the insertion of an ecotropic endogenous provirus, analogous to Ty elements in yeast and to gypsy and copia elements in Drosophila (Copeland et al., 1983). The d phenotype has been shown to be suppressed by a recessive, non-linked mutation at the dilute suppressor (dsu) gene. The dsu gene suppresses coat color mutations leaden and ashen whose mutant phenotypes are very similar to dilute, as well as suppressing the ruby eye color due to genes ruby-eye and ruby-eye-2. The exact mechanism of suppression is not understood, but it has been shown that dsu does not affect the transcription of the d gene (Moore et al., 1990).

In Drosophila melanogaster, many cases of intergenic suppression and enhancement have been reported. Modifiable alleles of most target genes have been shown to be of spontaneous origin (Green, 1955, 1956). Molecular analyses of these genes revealed that most spontaneous mutations in Drosophila are caused by the insertion of transposable elements at the
mutant locus (Levis et al., 1984). Most suppressor mutations involve a
gene whose product acts in trans to affect the transcription of the
transposable element located within the target locus. Most of the
transposable elements in Drosophila belong to three distinct classes: copia-
like-, foldback- and P- elements. All mobile elements involved in
intergenic suppression that have been studied at the molecular level belong
to the copia family, which includes copia and gypsy elements (Kubli,
1986). They all possess structural similarities and appear to be retroviral
in nature. The demonstration that transposable elements, especially the
copia-like transposable elements, reside at these modifiable loci, gave a
molecular basis for modifiable verses nonmodifiable alleles within one
locus. Examples of these suppressor-target loci systems are the suppressor
of Hairy wing (su(Hw)) which suppresses forked (f) yellow-2 (y2) and the
mutation Hw1 at the achaete-scute gene complex. The recessive loci
suppressor of sable (su(s)) suppresses the phenotype of sable (s), vermillion
(v), and purple (pr) and enhances the phenotype of lozenge1 (lZ) as well as
two bithorax alleles. The pr phenotype is also suppressed by suppressor of
purple (su(pr)). The phenotype of white-apricot (wa) is suppressed by
suppressor of wa (su(wa)) while the phenotype of wa is enhanced by
suppressor of forked (su(f)). The phenotype of f and some lZ alleles are
suppressed by su(f). These genes and their phenotypes are further detailed
in Table 1.

This study focuses on the actions of the suppressor of forked locus. The
suppression and enhancement properties, as well as other phenotypic
effects, of the many alleles of this gene will be discussed. To study, not
only $su(f)$, but other suppressor loci as well, many types of analyses are necessary. They range from the phenotypic interactions of the suppressor-target loci, to the functional studies of alleles, to the structural properties of the suppressor genes and their target loci. Therefore many approaches have been employed to better understand $su(f)$ and its actions in particular, and the genetics of suppressor systems in general.

Most of the historical findings and generalizations about $su(f)$ have been based on the genetics of very few of the $su(f)$ alleles. Even recent findings presented from different research groups tend to utilize very few of the alleles of $su(f)$. As shown in Lindsley and Zimm (1990) 28 alleles of $su(f)$ exist and are characterized in one way or another. The many phenotypes of $su(f)$ have by no means been examined for each of these alleles. Instead, a report of a particular $su(f)$ phenotype may be based on one or two alleles, while a second report concentrating on an entirely different phenotype, utilizes entirely different alleles. One of the purposes not only of this study, but for this research group is to present thorough genetic analyses of the majority of the $su(f)$ alleles, including their interactions and differences in phenotypic expressions. The beginning of these types of studies began with research on the $su(f)ts726$ allele (Russell, 1974) and are continued in this study. $Su(f)$ is a very important and complex gene, with many phenotypes. Its genetic interactions among its alleles and with target loci are only beginning to be understood.
History of su(f)

The suppressor of forked locus was discovered by M. Whittinghill in 1938. He isolated an X-ray induced, trans-acting, allele specific modifier mutation on the X chromosome, called su(f). It maps to the proximal portion of the X chromosome just distal of the heterochromatic / euchromatic border at map position 65.9. When the forked gene, map position 56.7, is mutant, bristles on the fly are thickened and somewhat gnarled at the ends, exhibiting the "forked" mutant phenotype. However, the phenotype of these same bristles return to near wild type in the double mutant f and su(f). However, as described by Green in 1955, suppression is allele specific at forked. The f^I, f^A, and f^5 alleles are suppressed by su(f), yet the f^3 and f^3N alleles are not. As stated above, an additional phenotype of su(f) is the enhancement of the white-apricot (w^a) mutation. White-apricot mutants show apricot colored eyes, but in the presence of su(f) show nearly white eyes (Green, 1959). Therefore, su(f) exhibits the suppressed or enhanced phenotype in combination with particular alleles at different target loci.

Transposable elements reside in both the modifiable alleles, f^I and w^a. A copia transposable element resides in one intron of the white gene causing the w^a phenotype (Goldberg et al., 1982, Levis et al., 1982). When su(f) is mutated, fewer full length white transcripts and more truncated transcripts are produced due to a premature transcription termination signal located in the 3' long terminal repeat (LTR) of the element. Thus the enhanced phenotype is a result of a reduction in the
amount of functional \textit{white} product (Kubli, 1986). A \textit{gypsy} transposable element is inserted in the RNA coding region of the \textit{forked} allele, $f^I$. The insertion of the \textit{gypsy} element results in a decrease in the amount of \textit{forked} encoded RNA transcripts presumably producing the \textit{forked} bristle phenotype. Mutations at \textit{su(f)} in combination with $f^I$, restore normal levels of these transcripts resulting in near wild type bristles. That \textit{su(f)} is affecting the \textit{gypsy} element is demonstrated by the finding that mutations at \textit{su(f)} do not affect the transcription of the wild type \textit{forked} gene, and do result in an increase in the accumulation of \textit{gypsy} RNA. The LTRs of the \textit{gypsy} element contain a TATA box and are part of promoter elements which are under developmental control. The expression of the \textit{gypsy} RNAs is maximal at the same developmental stage during which the \textit{forked} RNAs are expressed (Parkhurst and Corces, 1985). The suppression of the \textit{forked} mutant phenotype by \textit{su(f)} takes place at a transcriptional level. Therefore, the \textit{su(f)} locus encodes a trans-acting factor which regulates transcription of the \textit{copia} element and the \textit{gypsy} transposable element.

\textit{Suppressor of Hairy wing}, \textit{su(Hw)}, in \textit{Drosophila} also suppresses the \textit{forked} mutation, $f^I$. However, the transcription of the \textit{gypsy} element in $f^I$ is reduced in the presence of \textit{su(Hw)} mutations (Parkhurst and Corces, 1986). Thus \textit{su(f)} and \textit{su(Hw)} appear to have opposite effects on the transcription of the \textit{gypsy} element. The \textit{yellow$^2$} mutation, due to an inserted \textit{gypsy} element in the noncoding region of the \textit{yellow} locus, is suppressed by \textit{su(Hw)} but is not affected by \textit{su(f)}. This indicates that the wild type product of both \textit{su(f)} and \textit{su(Hw)} interfere actively with the transcription of \textit{gypsy} elements although showing opposite effects,
suggesting that more than one mechanism of modifier gypsy interaction is operating.

As discussed above, another suppressor gene in *Drosophila*, suppressor of white-apricot, *su(w<sup>a</sup>)*, like *su(f)*, acts on the transcription of the *copia* element at *w<sup>a</sup>*. Unlike *su(f)*, *su(w<sup>a</sup>)* increases readthrough of the 3' LTR of the *copia* element, resulting in more full length *white* transcripts, producing more wild type eyes (Mount et al., 1988). Suppression of *w<sup>a</sup>* results from an increase in functional *white* product. *Su(w<sup>a</sup>)* is another example of a trans-acting transcriptional modifier of *w<sup>a</sup>* showing an opposite effect to *su(f)*. However, not all mutations caused by the insertion of *copia* and gypsy transposable elements are modifiable. Thus, the type of element, site of its insertion, and nature of the mutated locus are potential factors in determining if an allele is modifiable by a given modifier gene.

The phenomena of suppression are widespread, however, the several suppressor systems that are known and characterized are not all alike. For example in bacteria and yeast, suppression is based on the production of a missense tRNA by the suppressor locus (Biswas and Gorini, 1972). In *Drosophila*, various alleles of the suppressor loci, *su(Hw)* and *su(f)* are recessive and act as null mutations. Thus any mechanism of suppression based on the production of a tRNA by the suppressor locus, such as the case of translational nonsense suppression in bacteria, can be eliminated as a possible explanation for this type of suppression. Also these genes are not located in the regions coding for tRNA which further eliminates the possibility that the molecular mechanism of suppression in *Drosophila* is based on modified tRNAs.
Some \textit{su(f)} alleles have, in addition to their effects on the transcription of transposable elements, phenotypes which are not the result of a transposable element insertion at a target locus. For example, several temperature-sensitive recessive lethal alleles of \textit{su(f)} have been isolated and found to be lethal in all genetic backgrounds. Two such alleles are \textit{su(f)\textsc{ts}67g} and \textit{su(f)\textsc{ts}726} (Dudick et al., 1974, and Russell, 1974). Both of these mutants survive and exhibit the suppressed forked phenotype at low or permissive temperatures (21°-25°C) while showing lethality at the high or restrictive temperatures (29°-30°C). Individuals of the \textit{su(f)\textsc{ts}67g / su(f)\textsc{ts}726} genotype survive and suppress \textit{forked} at the low temperature. At the high temperature they fail to complement each other's lethality. This indicates that both mutants are defective in a single vital function referred to as the lethal 67g function. The temperature sensitive period (TSP) of lethal 67g ranges from the first larval instar to the early pupal stage. Sublethal exposures of \textit{su(f)\textsc{ts}726} to the restrictive temperature result in a range of phenotypes from duplications or deficiencies of structures to pattern triplications, all dependent upon the time within the TSP exposure to the restrictive temperature occurred (Girton, 1983). The mutant \textit{su(f)\textsc{ts}67g} has been shown to affect the production of the hormone ecdysterone which is in turn essential for the transcription of the glue protein genes in the third instar (Hansson and Lambertsson, 1983). The induction of pattern abnormalities including duplications, triplications, and deletions in adult structures occurs in the temperature-sensitive (\textsc{ts}) allele, \textit{su(f)\textsc{ts}726}. This effect is thought to result from the induction of small patches of localized cell death in the imaginal discs at different
developmental stages (Girton and Russell, 1980, and Girton, 1981). These additional phenotypes imply that \textit{su(f)} has a function beyond that of regulating transcription of transposable elements.

A second vital function in \textit{su(f)} is shown by the \textit{su(f)}^p\textit{b} allele, a strong recessive lethal. \textit{Su(f)} \textit{pb} is defective for a vital function referred to as lethal \textit{pb}. Individuals with the \textit{su(f)}^p\textit{b} / \textit{su(f)}^ts678 or \textit{su(f)}^p\textit{b} / \textit{su(f)}^ts726 genotypes survive at all temperatures and exhibit suppression of \textit{forked} or enhancement of \textit{w^a}. The complementation of the lethal function without complementation of other functions is characteristic of a complex locus. Therefore, the two lethal functions in \textit{su(f)} represent two separable functional domains.

As previously stated, suppression and enhancement are the two other functions of \textit{su(f)}, and represent separable functional domains. This has been shown by analysis of several allelic combinations at various temperatures (J. Girton, Department of Genetics, Iowa State University, personal communication). For example \textit{su(f)}^p\textit{b} / \textit{su(f)}^ts678 individuals show complete suppression of \textit{forked} at 29°C but weak suppression at 21°C, while \textit{w^a} enhancement increases with the decreasing temperature. Individuals with the \textit{su(f)}^p\textit{b} / \textit{su(f)}^ts726 genotype show the same pattern of \textit{forked} suppression but weak \textit{w^a} enhancement at all temperatures. Individual of the \textit{su(f)}^t / \textit{su(f)}^ts726 genotype show increasing \textit{w^a} enhancement from 29°C to 21°C while the strong suppression of the bristles remains constant. The \textit{w^a} enhancement for the \textit{su(f)}^p\textit{b} / \textit{su(f)}^t individual also increases with increasing temperature, but the bristle suppression decreases with increasing temperature. Homozygous \textit{su(f)}^t
individuals show strong *forked* suppression and strong enhancement of $w^a$ at all temperatures. Individuals of the *su(f)*$^{pb}$/Y genotype show very little $w^a$ enhancement but show strong $f$ suppression (J. Girton, personal communication). Therefore, individuals with different combinations of *su(f)* mutations reared at different temperatures can have weak enhancement of $w^a$ and strong *forked* suppression, or strong $w^a$ enhancement and strong *forked* suppression, or strong enhancement of $w^a$ and weak *forked* suppression. These two effects, suppression and enhancement, appear to vary independently with different mutations.

**Recent Findings**

Recent work has helped to further the knowledge of the complexity of the *su(f)* locus and its interactions. However, very little work has been published, even recently, which discusses the complex genetics of *su(f)* and other suppressor loci. These studies have focused on the interaction of the suppressor loci with their target genes and the transposon transcriptional regulation involved for *su(f)* and other suppressor loci. No published report contains an in-depth comparison of *su(f)* alleles, such as the findings that are presented throughout this study. Most of the findings reported in the literature have concentrated instead on the basic mechanism of *su(f)* suppression and enhancement and not the genetics of the locus itself. Research in this group has focused not only on the genetics of the locus using complementation studies as a tool but its lethal phenotypes as well. The results from the complementation studies that are presented above have
provided many of the working hypotheses of this locus. Findings generated on the phenotype of the lethal alleles are presented below.

A phenotype of the lethal alleles is that they block or suppress the production of the hormone ecdysterone. When ts lethal alleles have been raised at the restrictive temperature during the third instar larval period, the normal, premolt rise in the ecdysterone level is blocked or suppressed (Hansson et al., 1981). This block of hormone production results in the alteration of transcription of several genes, including the glue protein loci. Northern analyses of RNA isolated from individual mutations for two su(f) alleles, su(f)\(^I\) and su(f)\(^ts67g\), and wild type was performed. The larvae were shifted to the restrictive temperature, 29°C, at the 2/3 molt, and RNA isolated 36, 42, and 48 hours later. These RNAs were probed with DNA from the sgs-3 glue protein locus. The size of the transcript which hybridized is 1.4 kilobases, kb, correlating to the glue protein RNA. It was transcribed at all times analyzed in the wild-type and su(f)\(^I\) flies. However, the transcript is not found in the su(f)\(^ts67g\) RNA at any time between 36 to 48 hours past the 2/3 molt. This result is also true of the ts allele su(f)\(^ts726\). Thus viable alleles of su(f) do not block the transcription of glue protein RNA while the ts lethal su(f) alleles do block the transcription (L. Girton, Department of Genetics, Iowa State University, personal communication). Unlike suppression and enhancement, this effect is not dependent on a mutation at a second site which strongly suggests that su(f) does play a role in normal development. This block in transcription has been shown to have an indirect effect on the induction of glue protein gene transcription. The transcription of the glue protein gene can be
induced when exogenous ecdysterone is administered to hormone deficient larvae which carry a lethal $su(f)$ mutation thus rescuing their lethality. However, whether the involvement of the $su(f)$ product is primary or secondary on the presence of the ecdysterone hormone is not known (Hansson and Lambertsson, 1983).

Additional data has been reported which helps to explain mechanisms of $su(f)$ action. The gypsy element present in the $f^I$ allele was completely sequenced (Marlor et al., 1986). It is approximately 7.5 kb in length and codes for three putative protein products. The LTRs are 482 base pairs, bp, long and contain transcription initiation and termination signals. The sequences are homologous to the polypurine tract and tRNA primer binding site of retroviruses and are located adjacent to the LTRs. The central region of the element contains three different open reading frames, the second of which codes for a putative protein. This putative protein has high homology to retroviral proteins, including gag-specific protease, reverse transcriptase, and DNA endonuclease (Marlor et al., 1986). The finding of two sequences, within the gypsy element, which bind proteins were reported later by Mazo et al. (1989). One of these sequences is an imperfect palindrome which is homologous to the $lac$-operator of $E. coli$, while the other contains a reiterated sequence homologous to the octamer that is the core of many enhancers and upstream promoter elements. Deletion mutants have shown that these DNA regions are negative and positive regulators of transcription. Also reported was the finding that binding to a negative regulator (silencer) is weakened in nuclear extracts isolated from fly stocks carrying $su(f)$ mutations which activate gypsy
transcription. Therefore the *su(f)* gene seems to code for a protein capable of gypsy repression.

Recent reports have added to the understanding of the enhancement of *w^a* as well. The pattern of *w^a* transcription was detailed by Birchler and Hiebert, 1989. The inserted *copia* element is transcribed in the same direction as the *white* gene. The majority of RNAs are initiated at the 5' start site of *white* and terminate in the 3' LTR of *copia*. A low level of transcription proceeds to the 3' terminus of *white* and the *copia* sequences within the second intron are spliced out. This low level of functional message accounts for the leaky phenotype of *w^a*. However, there are RNAs that initiate in the 5' LTR of *copia* and terminate in the 3' terminus of *white*, as well as those that initiate in the 5' start of *white* and terminate in the 5' LTR of *copia*. There are also messages that initiate in the 5' *white* start which are intermediate in size to the full length message and the one terminated in the 3' *copia* LTR. The *Enhancer of w^a* mutation reduces the level of RNA that is of normal size for *white* (2.6 kb) as well as the product which initiates in *copia* and terminates at the 3' terminus of *white*. The RNAs that are transcribed through the 3' LTR of *copia* under normal conditions are reduced in quantity by *E(w^a)*. Since the function of *su(f)* and *E(w^a)* are so similar at *w^a*, it is hypothesized that *su(f)* also acts to reduce the quantity of RNAs present that are transcribed through the 3' LTR of the *copia*.

The molecular work that has been completed on *su(f)* has not been formally reported. The *su(f)* gene was cloned by transposon tagging using a P element insertion mutant, *su(f)^MS252* (also called *su(f)^hd252* in this
study) (K. O'Hare, Imperial College, London, England, personal communication). The insertion strain was generated by M. Simmons (University of Minnesota). The region has been extended to 50 kb with a Sal I site close to the insertion of the P element as the origin for DNA coordinates. Breakpoints within this region were identified by L. Kelly (University of Melbourne) in order to orient the clone with respect to position on the chromosome. As shown in Figure 10 the positive coordinate values are toward the centromere while negative values are toward the telomere. Many $su(f)$ alleles were molecularly analyzed by this group and the findings will be discussed later in this study. A major portion of this study was to use this clone to also molecularly analyze many $su(f)$ alleles which will be discussed later. Three major RNAs were identified by RNA blotting and cDNA analysis. They are encoded in the interval -0.3 to +3.7. The size of the first transcript is 1.3 kb comprising 3 exons, while the second and third transcripts are 2.6 kb and 2.9 kb, each comprised of the same 8 exons (including the 3 exons of the first transcript). These transcripts predict 2 proteins (Lindsley and Zimm, 1990).

Recent developments on other suppressor genes have been reported on suppressor of sable ($su(s)$), a newly discovered locus Darkener-of-apricot (Doa), and the alcohol dehydrogenase gene (adh) as a target gene for $su(f)$ modification. The $su(s)$ gene was cloned by Voelker et al. (1989). It has a 5 kb message and has ten genetic complementation groups within the region. Suppression is seen at second site mutations caused by insertions of the mobile element 412 (a member of the copia-family). Mutations at the
Doa locus suppress the \( w^a \) phenotype and enhance the \( w^{sp55} \) phenotype. These mutations are dominant suppressors and enhancers, and all alleles are recessive lethals. Rare homozygotes show extreme suppression of \( w^a \) and extreme enhancement of \( w^{sp55} \). The \( w^{sp55} \) allele is due to a transposon insertion because it is known to possess LTRs. However, its restriction map is different from that of \textit{copia} or any other described \textit{Drosophila} transposable element. This locus appears vital to the fly since one phenotype is its recessive lethality, a suppressor loci phenotype previously unique to \( su(f) \) and \( su(Hw) \) (Rabinow and Birchler, 1989).

The report on the \( adh \) target gene involves a variant \( adh \) allele (RI-42) which contains a \textit{copia} insertion 240 bp upstream from the distal \( adh \) start site (in the adult). Levels of \( adh \) transcripts are reduced in tissues and at life stages where \textit{copia} is actively expressed and are affected in trans- by mutant alleles at the \( su(w^a) \) and \( su(f) \) loci. These suppressors have no effect on \( adh \) expression in wild-type \textit{Drosophila}. However, loss of function alleles at \( su(f) \) result not in the expected loss of \( adh \) transcription, showing an enhanced mutant phenotype, but result in an increase in levels of \( adh \) expression, giving partial suppression of the mutant phenotype (Strand and McDonald, 1989).

These recent findings, as well as those presented earlier have helped to formulate a hypothesis as to how the \( su(f) \) product acts to modify second site mutations. Other suppressor loci are phenotypically similar to \( su(f) \). \textit{Doa} shows both a strong suppression and enhancement effect as well as recessive lethality. \textit{Suppressor of sable} also shows enhancement of some target genes and suppression at others. These modifiable alleles are due to
the insertion of a transposable element, most often a member of the copia-family of transposable elements. The transcription of the gene in which the transposable element is located is not affected by mutations at these suppressor loci. Only the transcription of the transposable element is affected. In the case of mutations at su(f), the transcription at the gypsy element is activated, while transcription at the copia is terminated. This suggests the the wild-type product of su(f) ,su(f)+, is a protein capable of gypsy repression (Mazo et al., 1989) while it acts as an activator of copia transcriptional readthrough. This hypothesis seems to hold true to each case discussed except for the adh allele, above. One important point made in the literature is that the position of insertion of the transposable element has a strong effect on the resulting phenotype of the modified locus. It is possible that mutations at su(f) are acting in the same manner on the copia element in adh RI-42. However, due to the position of the copia insert 240 bp upstream of the transcriptional start site of adh, a profoundly different effect on adh transcription and thus its phenotype is seen.

Model of su(f) Action

What is the mechanism of su(f)+ action? When su(f) is mutant, it suppresses the phenotype of certain f and lz alleles, and enhances w^a and certain lz alleles. It shows recessive lethality when ts alleles are raised at restrictive temperatures. This lethality affects the transcription of glue protein gene, thus affecting the production of the ecdysterone hormone. The induction of pattern abnormalities is seen in the su(f)ts678 and
su(f)ts726 alleles at restrictive temperatures. Neither the glue protein transcription effect nor the pattern abnormality effect are dependent on mutations at other loci. This complex gene has many phenotypic effects. It produces at least three transcripts and possibly two proteins. The many results, often obtained from studies on many different su(f) alleles not only by researchers in other groups but those involved in this study as well, have led to the current hypothesis for the model of su(f)\(^+\) action.

Su(f) is a complex gene. Our current model for action of su(f) is based on the hypothesis that the gene consists of four functional domains. These domains include two domains responsible for lethality, one for the suppression effect, and one for the enhancement effect. The demonstration that two lethal conditions within one locus can complement each other and that the suppression and enhancement phenotypes are separate provides the foundation for this model. Figure 1 diagrams this model. One of the lethal domains is called lethal 67g and is referred to as L1. The second lethal domain is called lethal pb and is referred to as L2. The suppression domain is referred to as S1 while the enhancement domain is referred to as S2. These latter two domains act as regulators of transcription at the gypsy and copia elements respectively. Therefore, the separable functions of the two lethal conditions, suppression, and enhancement have led to the hypothesis of four domains each intimately involved with the action of this gene's product.

The mechanism of su(f)\(^+\) action is diagrammed by the binding of the putative su(f) protein directly to the DNA involved. The su(f) product is shown binding to the 3' LTR of the copia element in Figure 1. The
Figure 1. Current Model of the Action of the $su(f)$ Product
DMA trans-acting gene product(s)

transcription

"copia" transposable element

"gypsy" transposable element

? cis-acting enhancer/promoter

"Normal" Drosophila gene

transcription
binding is diagrammed this way because the transcription of the *copia* element within \(w^a\) is terminated at the 3' LTR when \(su(f)\) is mutated. Therefore \(su(f)^+\) activates the transcriptional readthrough of copia elements at target loci. The \(su(f)\) product is shown binding to the 5' LTR of the *gypsy* element in Figure 1. In this case the binding is diagrammed this way because when \(su(f)\) is mutant the level of *gypsy* transcription at *forked* is increased which increases the level of wild-type *forked* transcripts. The LTRs contain a TATA box and are part of promoter elements which are under developmental control. When \(su(f)\) is mutant, expression of the *gypsy* RNAs is maximal at the same developmental stage during which the *forked* RNAs are expressed. Therefore \(su(f)^+\) must act to negatively regulate the *gypsy* transcription. The suppression and enhancement effects have been shown to take place at the transcriptional level, whether this is a direct interaction of the \(su(f)\) product with the elements themselves is still not clear.

The \(su(f)\) product also indirectly interferes with the transcription of the *glue* protein gene. This interaction is diagrammed in Figure 1. It shows \(su(f)^+\) acting to suppress or block the transcription of a "normal" *Drosophila* gene. The mechanism of the interaction is not known which is why the \(su(f)\) product is diagrammed at both the promoter region and the downstream portion of such a gene. Each of the ts alleles phenotypically demonstrates this prevention of transcription, but when these alleles are heterozygous they complement each other's effect on the transcription, thus preventing the lethal phenotype. Thus the L1 and L2 functional domains must at least complement one another in the protein product to prevent
lethality as diagrammed. The S1 and S2 domains are shown as separate
domains. This model proposes that the suppression function and the
enhancement function are genetically separable events. Thus it proposes
that they are functionally separate domains within the protein product.
This separation of phenotypic effects has not been suggested, nor proven
before this research. Research on this locus, presented in the literature, has
usually been based on the suppression / enhancement effects of one very
strong $su(f)$ allele, $su(f)^I$. This study focuses on many $su(f)$ alleles, their
complementation patterns, and their varied phenotypes with respect to these
two effects. The complementation studies as well as the search for
molecular analyses conducted among the many $su(f)$ alleles, therefore, have
provided the foundation for this hypothesis. This question is of central
importance to this study and will be more completely answered after the
results have been presented and discussed.

Finally why does a gene product which seems to have a vital function
also show such distinctive phenotypes which are solely due to its regulation
of transposable element transcription? It is possible that $su(f)^+$ acts as a
regulator of transcription of $Drosophila$ genes and has been adapted by
transposable elements. Another alternative is that $Drosophila$
transcriptional regulatory genes have evolved into regulators of
transposable elements as a defense mechanism. The normal function of
$su(f)$, therefore, could have one or more different mechanisms of action.
To achieve better understanding of this modification, even more
information about the structure and function of modifier genes and their
target sites will be required.
Objectives of Research

The genetic experiments that are presented were designed to test the model of action of \textit{su(f)} and answer questions it has presented. The genetic separability of the suppression and enhancement effects was a primary goal of this study. To accomplish this, a complementation study was completed. This study allows for the detailed observation of many \textit{su(f)} alleles in different allelic combinations and each genotype's affect on the suppression phenotype and the enhancement phenotype. In addition this study allows for the determination of allelism of several new \textit{su(f)} mutations as well as completing the in-depth comparative allele study. The studies on the \textit{lz} locus were also designed to test the question of genetic separability of suppression and enhancement. The purpose is to determine how \textit{su(f)} affects the phenotype at a single locus. Several spontaneous mutation alleles, at least one of which is due to the insertion of a \textit{gypsy} element, were tested for a suppressed or enhanced phenotype when \textit{su(f)} is mutant. It also helped to better analyze the effects of suppression and enhancement at one locus by examining a single phenotype. Allele tests of new \textit{su(f)} mutations were also conducted to test their suppression and enhancement effects in new background as well as to thoroughly document their phenotype.

Mutagenesis experiments were conducted in order to obtain new and different phenotypes of \textit{su(f)}. These experiments were planned in conjunction with the molecular analyses of many \textit{su(f)} alleles. These alleles
were examined to determine if they contained any differences in their restriction fragment lengths when compared to wild type. The purpose of this experiment is to correlate the molecular lesion with the phenotype of this allele. Since the model proposes 4 functional domains, it was the purpose of this experiment to determine if, by molecular analyses of the alleles, a physical relationship of lesion to functional domain could be established. If these molecular lesions could be correlated to any particular phenotype: lethality, suppression, or enhancement a better understanding of the fine structure of this locus could be established. Finally, in situ hybridization of cloned su(f) DNA to salivary gland chromosomes was conducted to determine if the cloned su(f) fragments hybridized to the expected su(f) chromosome position.

The research proposed will yield much needed data on the su(f) gene and on the different su(f) alleles. It will also address the complicated genetic interactions of these alleles, as well as their suppression and enhancement effects at other loci. Most importantly, it will address the question of suppression and enhancement as genetically separable functions. This information will help to refine the current hypothesis and expand our current understanding of trans-acting transcriptional modification.
MATERIALS AND METHODS

Genetic Experiments

Culture techniques and stocks
All cultures of Drosophila melanogaster were maintained on a standard medium consisting of cornmeal, sugar, agar, mold inhibitor and live yeast. Cultures for both experimental purposes and for stocks were kept in quarter-pint glass bottles, while single pair matings were kept in small glass vials. Stocks were maintained at room temperature (RT) of approximately 23°C while experimental crosses were maintained in temperature controlled incubators ranging from 21°C to 29°C. The cultures which served as stocks were changed every 2 weeks. The parents used in experimental crosses were generally subcultured to a second bottle after 4 days at 25°C, then to a third bottle after an additional 4-6 days, and then discarded. (The subculture time varied for crosses at higher or lower temperatures.) Virgin females for experimental crosses were collected and kept separate for 4-5 days at RT (or up to 7 days at 21°C) before mating. Males were kept separate for at least one day before mating. Stocks for all crosses were originally obtained from Dr. Jack Girton, Dr. Bill Welshons, the University of California at Davis, Department of Genetics Stock Center, the Mid-America Drosophila Stock Center at Bowling Green State University, or were generated in the lab. Tables 1 and 2 contain a list and description of all markers and chromosomes used throughout the genetic experiments.
<table>
<thead>
<tr>
<th>Symbol / Full Name</th>
<th>Chromosome</th>
<th>Map Location</th>
<th>Phenotype / References</th>
</tr>
</thead>
<tbody>
<tr>
<td>yellow-2</td>
<td>X</td>
<td>0.0</td>
<td>yellow body and wings, dark bristles</td>
</tr>
<tr>
<td>su(s) suppressor of sable</td>
<td>X</td>
<td>0.0</td>
<td>suppresses several alleles, s, v, pr, sp</td>
</tr>
<tr>
<td>su(w') suppressor of w'</td>
<td>X</td>
<td>0.1</td>
<td>suppresses certain alleles of w'</td>
</tr>
<tr>
<td>w' white-apricot</td>
<td>X</td>
<td>1.5</td>
<td>light orange colored eyes</td>
</tr>
<tr>
<td>N Notch</td>
<td>X</td>
<td>3.0</td>
<td>N/N lethal; N/+ wing notch &amp; thick veins</td>
</tr>
<tr>
<td>cv crossveinless</td>
<td>X</td>
<td>13.7</td>
<td>crossveins absent</td>
</tr>
<tr>
<td>ct cut</td>
<td>X</td>
<td>20.0</td>
<td>incised wing margins</td>
</tr>
<tr>
<td>lz lozenge</td>
<td>X</td>
<td>27.7</td>
<td>ovoid, glossy, and spectacle eyes</td>
</tr>
<tr>
<td>v vermillion</td>
<td>X</td>
<td>33.0</td>
<td>bright scarlet eye color</td>
</tr>
<tr>
<td>car carnation</td>
<td>X</td>
<td>62.5</td>
<td>dark, ruby eye color</td>
</tr>
<tr>
<td>f forked</td>
<td>X</td>
<td>56.7</td>
<td>bristles, short, gnarled, bent at ends</td>
</tr>
<tr>
<td>B Bar</td>
<td>X</td>
<td>57.0</td>
<td>Narrow vertical eye shape</td>
</tr>
<tr>
<td>su(f) suppressor of f</td>
<td>X</td>
<td>65.9</td>
<td>suppresses f, enhances w'</td>
</tr>
<tr>
<td>bb bobbed</td>
<td>X</td>
<td>66.0</td>
<td>thin, short bristles &amp; etched abdomen</td>
</tr>
<tr>
<td>*Su(f) Suppressor of f</td>
<td>2</td>
<td>74.0</td>
<td>Dominant suppressor of f, not available</td>
</tr>
<tr>
<td>M(3)67C^4 Minute</td>
<td>3</td>
<td>28.9</td>
<td>Dominant-minute, small bristles (M(3)i^{55})</td>
</tr>
<tr>
<td>su(Hw) supp. Hairy wing</td>
<td>3</td>
<td>54.8</td>
<td>suppresses several alleles, y^2, Hw, lz, f</td>
</tr>
<tr>
<td>su(pr) supp. of purple</td>
<td>3</td>
<td>95.5</td>
<td>suppresses several alleles, pr, lz^{34}, f, Hw</td>
</tr>
</tbody>
</table>
Table 2. References and Description of Special Chromosomes and Stocks

<table>
<thead>
<tr>
<th>Symbol / Full Name</th>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM7  In(1)FM7b, w&lt;sup&gt;a&lt;/sup&gt;, B</td>
<td>X chrom. balancer, with w&lt;sup&gt;a&lt;/sup&gt; and B</td>
<td>Lindsley &amp; Zimm, 1990</td>
</tr>
<tr>
<td>ywflY Compound(1)Double X</td>
<td>attached X, for maintenance of X chromosome in males, y, w, and f</td>
<td>Lindsley &amp; Grell, 1968</td>
</tr>
<tr>
<td>DfVe738 Df(1)su(f)738</td>
<td>X chrom. def. covering su(f)</td>
<td>Schalet &amp; Lefevre, 1973</td>
</tr>
<tr>
<td>Dp1:2 B&lt;sup&gt;s&lt;/sup&gt; / Cy</td>
<td>Bar-stone dup. on 2L tip</td>
<td>Lindsley &amp; Zimm, 1987</td>
</tr>
<tr>
<td>Cy  In(2L) Curly</td>
<td>Inversion to balance chrom. 2</td>
<td>Lindsley &amp; Zimm, 1987</td>
</tr>
<tr>
<td>Dp 1:3 B&lt;sup&gt;s&lt;/sup&gt;</td>
<td>Bar-stone dup. on 3L tip</td>
<td>Lindsley &amp; Zimm, 1987</td>
</tr>
<tr>
<td>TM3 In(3LR)TM3, Sb</td>
<td>3rd chromosome balancer</td>
<td>Lindsley &amp; Zimm, 1990</td>
</tr>
<tr>
<td>B&lt;sup&gt;s&lt;/sup&gt; Y</td>
<td>Bar-stone dup. on Y, su(f)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Lindsley &amp; Grell, 1968</td>
</tr>
<tr>
<td>Ore R</td>
<td>wild type stock-origin-Oregon</td>
<td>Lindsley &amp; Grell, 1968</td>
</tr>
<tr>
<td>Can-S</td>
<td>wild type stock-orig.-Canton, OH</td>
<td>Lindsley &amp; Grell, 1968</td>
</tr>
</tbody>
</table>
EMS mutagenesis

The alkylating agent, ethyl methane sulfonate (EMS), is mutagenic when fed to adult Drosophila males. It was used to induce mutations throughout the genome of the males with the hopes of isolating a mutation in a particular location on the X chromosome by screening this chromosome in later generations. The following procedure generally follows one described by Lewis and Bacher (1968). Freshly emerged males of the genotype $y^2 w^a f^l / Y$ were isolated and starved for several hours. Filter paper attached to the bottom of an empty bottle was soaked with a solution of 0.025M EMS in 1% sterile sucrose. Approximately 50 males were transferred to the bottle containing the mutagen, then the bottle was stoppered with a disposable cotton plug. The males were allowed to feed on the EMS-sucrose solution for 24 hours in a fume hood at RT. The males were transferred from the bottle containing mutagen to a bottle with fresh food for several hours. All mutagen is deactivated in a solution of 4 grams (g) of NaOH dissolved in 100 milliliters (ml) H$_2$O and 0.5 ml of thioglycolic acid (mercaptoacetic acid). Deactivation of the mutagen takes place in approximately 12 hours.

Groups of 10 treated males were mated to 25 $y^2 w^a cr^6 f^l DefVE738 / FM7$ aged virgin females. The crosses were held at 23°C and the parents were subcultured every 4 days for 12 days then discarded. The treated chromosome is now represented as $y^2 w^a f^l \cdot$. In the G$_1$, only two genotypes of females were expected. The first genotype is $y^2 w^a f^l \cdot / y^2 w^a cr^6 f^l DefVE738$. These females are hemizygous for the region of the X chromosome uncovered by the VE738 deficiency which includes $su(f)$. 
If any new viable, visible *su(f)* mutations were induced, the females would show suppression of *forked* and enhancement of *w*\(^a\), the *su(f)* mutant phenotype. An advantage of this screen is the ability to detect *su(f)* mutations in the first generation. Females, showing the mutant phenotype, were mated to *FM7 / Y* males and a stock was established.

The second genotype of females expected in the G1 of this cross was *y\(^2\) w\(^a\) f\(^1\) / FM7*. These females were collected as virgins and used in a G2 mutation screen. They were mated to *y\(^2\) w\(^a\) cr6 f\(^1\) DefVE738 / B\(^8\)Y* males. The *B\(^8\)Y* carries a duplication of the proximal portion of the X chromosome that contains a wild type copy of the *su(f)* locus. Each female was mated to 3 males in small glass vials and the parents were discarded after 4 days in the vials. The expected G2 female progeny were *y\(^2\) w\(^a\) f\(^1\) / y\(^2\) w\(^a\) cr6 f\(^1\) DefVE738* and *y\(^2\) w\(^a\) f\(^1\) / FM7*. The expected G2 male progeny were *y\(^2\) w\(^a\) f\(^1\) / B\(^8\)Y* and *FM7 / B\(^8\)Y*. The females and males carrying the *FM7* balancer X chromosome were separated based on their phenotype and discarded. If the G2 females, heterozygous for *DefVE738*, showed *w*\(^a\) enhancement and/or *forked* suppression and were virgins, they were mated to *FM7 / Y* males. If there were no females heterozygous for the deficiency, the sibling *y\(^2\) w\(^a\) f\(^1\) / B\(^8\)Y* males were mated to *y\(^2\) w\(^a\) cr6 f\(^1\) DefVE738 / FM7* females, followed by a screen of females in the G3. From these crosses, stocks of *y\(^2\) w\(^a\) f\(^1\) / FM7 X FM7 / B\(^8\)Y* were established.

This G2 mutation screen will identify mutations of *su(f)* and mutations in the region uncovered by *DfVE738*. The design of this screen allows for a screen of viable mutations in both the G1 and G2, however, unlike the G1
screen, lethal mutations as well as viable mutations will be recovered from the G2 screen. Also by mating single G1 females, individual treated chromosomes are tested for mutations.

**TEM and DEB mutagenesis**

These mutagenesis experiments are designed to screen large numbers of G1 progeny and hopefully will result in the generation of suppressor of *forked* intragenic deletions. The mutagen 1X triethylenemelamine, TEM (purchased from Polysciences, Inc.) is used to induce small deletion mutations throughout the genome of *Drosophila* males. The mutagenesis follows the procedure as described by Grigliatti (1986). Freshly emerged males of the genotype *y2 w1 f1 y+* were collected from the homozygous stock and aged 2-4 days. TEM is a powder and is made up in a sterile 1% sucrose solution to a final concentration of 0.15mM. Two circles of Whatman paper were cut to fit and attached to the bottom of half-pint bottles. Several concentrations of the TEM-sucrose mutagen were tested ranging from 0.2X to 2X. Groups of 100 males were transferred to a bottle stoppered with a disposable cotton plug. The mutagen was then drawn into a 10cc syringe, inserted through the stopper, and expelled onto the filter while the males were near the top of the bottle. The flies were allowed to feed on the TEM-sucrose overnight (O/N) or up to 24 hours. The flies were then transferred to a bottle with fresh food and allowed to feed and recover O/N. All mutagen was deactivated in a solution of 4 g of NaOH dissolved in 100 ml H2O and 0.5 ml of thioglycolic acid
(mercaptoacetic acid). Deactivation of the mutagen takes place in approximately 12 hours.

Another mutagenesis experiment was completed using the liquid mutagen diepoxybutane, DEB, purchased from Sigma. DEB also induces small deletions throughout genome of *Drosophila* males. Freshly emerged males of the genotype *y*² *w¹*¹ *f¹*¹ *y*² were collected from the homozygous stock and aged 2-4 days. The mutagen was added to a solution of 1% sucrose for a final concentration of 0.006M, according to Leicht and Bonner, 1988. The sucrose-mutagen solution was made by adding 0.0504 ml (or 50.4 ul) of DEB to 100 ml of sterile 1% sucrose. The mutagen solution was then administered and deactivated as described above. One "set" of mutagenized males consisted of eight bottles of mutagen holding 100 males each. Two sets of 800 mutagenized males were mated and subcultured.

In both mutagenesis experiments, approximately 20 to 25 treated males were mated to 50 *y*² *w¹*¹ *f¹*¹ *su(f)¹* aged virgin females collected from the homozygous stock. The cross was maintained at 21°C. In the TEM mutagenesis, the males were subcultured every 2 days to a bottle with a new group of females. The bottles were labelled "a", "b", "c", and "d". The males were transferred to "d" after 3 days. The mated females and remaining parents in "d" were allowed to remain in the bottles for 8 days then discarded. However, in the DEB experiments, the males were mated and subcultured after four days, then all parents cleared after eight more days. A total of 128 bottles were set up for the DEB experiment. Both sets of mutagenesis utilize the same cross described as follows.
The expected G1 progeny were $y^2 \text{wa}^f l^* \cdot y^+ / y^2 \text{wa}^f l^* \text{su}(f)^l$ females and $y^2 \text{wa}^f l^* \text{su}(f)^l / Y$ males. The heterozygous females were scored for the suppressor of forked phenotype, that is, enhancement of white-apricot eyes and suppression of forked bristles. This visible screen is possible because every known allele of suppressor of forked fails to complement with $\text{su}(f)^l$. Lethal $\text{su}(f)$ alleles (including deficiencies) are visible when heterozygous with $\text{su}(f)$. This allows the G1 screening of all possible $\text{su}(f)$ mutations, including lethals mutations. Any female which shows the characteristic $\text{su}(f)$ phenotype and also carries the $y^+$ marker will be deemed a "potential mutant" and will be single pair mated in the G2 generation.

The $y^2 \text{wa}^f l^* \cdot y^+ / y^2 \text{wa}^f l^* \text{su}(f)^l$ G1 females showing the $\text{su}(f)$ phenotype were singly mated to three or four $y^2 \text{wa} \text{ct}^6 f^l \text{DefVE738} / B^s Y$ males. The expected G2 female progeny were $y^2 \text{wa}^f l^* \cdot y^+ / y^2 \text{wa} \text{ct}^6 f^l \text{DefVE738}$ and $y^2 \text{wa}^f l^* \text{su}(f)^l / y^2 \text{wa} \text{ct}^6 f^l \text{DefVE738}$. The expected males are $y^2 \text{wa}^f l^* \cdot y^+ / B^s Y$ and $y^2 \text{wa}^f l^* \text{su}(f)^l / B^s Y$. The females and males not carrying the $y^+$ marker are discarded since they do not carry the treated chromosome. The females were used for comparison purposes since they showed the $\text{su}(f)^l$ phenotype. The $y^2 \text{wa}^f l^* \cdot y^+ / y^2 \text{wa}^f l^* \text{su}(f)^l$ females were screened for the $\text{su}(f)^l$ phenotype. Viable, visible mutations showed the $\text{su}(f)^l$ phenotype. If this class of progeny was absent, the sibling $y^2 \text{wa}^f l^* \cdot y^+ / B^s Y$ males were used to establish a stock and further analyze the potential mutation.

Two control crosses were done for these mutagenesis experiments. First, untreated males, allowed to feed in a bottle with 1% sucrose only,
were crossed and subcultured the same as the treated males. The untreated males in the "control cross" allowed the determination of the "normal" number of progeny per bottle. This cross also showed that there were no preexisting mutations in the stock. A second control cross was to mate a group of treated males to C(1)DX / Y females (also called y w f females). The resulting progeny were triplo-X females (of which only a few survive), Y / Y, which is lethal, C(1)DX / Y females, which serve as the "normal" number of females expected, and y2 w a fI* · y+ / Y males. If the correct dose of mutagen was used, these males should show at least a 50% lethality. This control cross allowed the number of lethal hits to the X chromosome to be measured. A final control was the y+ marker carried by potential mutant females in the G1 distinguishing them from any y2 w a fI su(f)I females exhibiting the su(f) phenotype due to a nonvirgin female parent.

**Single P element mutagenesis**

Mutagenesis using a single P element as the mutagen was also attempted. A goal of this mutagenesis was to obtain a P element insertion in su(f).

During this "controlled" mutagenesis, two types of P elements were employed. The first, called P(ftz) 123.1, was the "mutator". It contained the ftz promoter and the E. coli β gal gene to be used as a marker. However, this element could not excise and transpose autonomously, since it no longer contained the sequences for transposase production. The second P element, known as the "jumpstarter", employed in this scheme was called PΔ2-3 "wings clipped". Its function was to produce transposase
in order for the P123.1 element to transpose. The PΔ2-3 element did not transpose since it lacked cis-acting sequences necessary for mobilization (Cooley et al., 1988b). The stocks containing the P elements used in this mutagenesis were kindly provided by Dr. Spyros Artavanis-Tsakonas.

The mutagenesis was achieved by crossing the two strains of Drosophila containing the two types of P elements together. This created a hybrid dysgenic first generation within which the "jumpstarter" was producing transposase and the "mutator" was transposing. These first generation flies were then outcrossed in order to separate the two P elements. Mutations at su(f) were screened in the second generation. To create the chromosomes with all the appropriate markers for this mutagenesis, many genetic crosses were done. It was necessary during the construction of these chromosomes to make certain that the P123.1 element was still present. Its presence provided no external phenotype in the adult, so embryos were stained with an X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) solution. If P123.1 was present in the genome, a blue coloration of the embryo due to the β gal sequences would result. This mutagenesis involved very complicated genetic crosses necessary to create chromosomes with both P elements and the correct genetic markers in order to follow the progress of the hybrid dysgenesis. These crosses are diagrammed in Appendix 1.

**Complementation studies**

Complementation studies are conducted in order to determine whether mutations are actually alleles. The purpose of this complementation study was not only to determine allelism of several su(f) mutations but to
determine how different phenotypic effects of known su(f) alleles interact. The alleles studied were su(f)+, su(f)1, su(f)94, su(f)100, su(f)R617, su(f)ts726, and su(f)ts67g. These alleles were studied in both the homozygous and heterozygous condition at three different temperatures, 29°C, 25°C, and 21°C. The first step was to study the alleles in the homozygous condition. Females and males were selected from homozygous stocks with the markers y2, wR, and f1 as well as one of the su(f) alleles already listed. The exception is the temperature sensitive allele, su(f)ts726 which utilized the cross: y2 wR f1 / su(f)726 X FM7 X y2 wR f1 / su(f)726 / Y to generate homozygous females for each temperature regime. Parents for these crosses were 25 females, nonvirgins, and 15 males. The bottles containing the parents of the same genotype were placed at each of the three experimental temperatures. The bottles at 29°C were subcultured to "b" after 3 days and the parents cleared from "b" after another 3 days. The subculturing and clearing took place after 4 days at 25°C, and after 5 days at 21°C. The homozygous females were counted and scored for eye color, i.e., the level of white-apricot enhancement, and for the number of forked bristles, i.e., forked suppression. Scoring generally took place on Day 13 for 29°C, Day 16 for 25°C, and Day 21 for 21°C. The scoring procedure is discussed in the Results Section.

The next step was to determine the phenotype of various heterozygous combinations of su(f) alleles. This was done by collecting females heterozygous for two different su(f) alleles from the list above. The heterozygous su(f) allele combinations studied were: su(f)94 / su(f)1, su(f)94 / su(f)ts726, su(f)94 / su(f)ts67g, su(f)100 / su(f)1, su(f)100 /
These heterozygotes were constructed by mating aged virgin females carrying one $su(f)$ allele to males carrying the other $su(f)$ allele. Virgin sources were stocks carrying the alleles, $su(f)^{94}$, $su(f)^{100}$, $su(f)^{R617}$, and $su(f)^+$. Male sources were stocks carrying the alleles, $su(f)^1$, $su(f)^{ts726}$, $su(f)^{ts678}$, and $su(f)^{94}$ (for the $su(f)^+ / su(f)^{94}$ heterozygote). The parents for these crosses were mated, subcultured, and cleared exactly the same as the homozygous parents. These crosses were also made at the same three temperature regimes and scored according to the same time schedule.

After each genotype was scored and the results recorded, a picture was taken both of the eye phenotype and of the thoracic bristles, demonstrating the enhancement and suppression effects respectively. A 35mm camera was loaded with Kodak™ Ektachrome 160 color slide film. The camera was mounted onto a dissecting microscope camera attachment and pictures were taken. The F-stop used was 2.0, while the shutter speed varied from 30 to 15 to 8 depending on the magnification. The magnifications used were 4X and 6.3X. The resulting homozygous and heterozygous phenotypes were photographed and are presented in the RESULTS section.

**Tests of $su(f)$ with the lozenge gene**

A test of the enhancement and suppression abilities of $su(f)$ on a series of $lz$ alleles was conducted. The $lz$ alleles tested were $lz^1$, $lz^{34}$, $lz^{36}$, and $lz^{37}$. All of the stocks except $lz^1$ were homozygous for that particular $lz$
allele and did not carry other markers. The $lz^I$ stock carried many other markers on the X chromosome, so a recombination experiment was performed to remove the other markers and establish a stock with $lz^I$ alone. The stock was $y^2 w^a cl^6 f^I lz^I v / Y X y w f$. These males were mated to wild type females ($+/+$) and heterozygous virgin females ($y^2 w^a cl^6 f^I lz^I v / +$) were collected in the next generation. These females were mated to wild type ($+/Y$) males. Recombinant males were screened in the next generation. A recombinant that carried the $lz^I$ marker alone was never isolated, instead a recombinant with the $lz^I$ and $v$ markers was isolated, and a stock established.

The next step was to construct a series of chromosomes each carrying one $su(f)$ allele in cis with one $lz$ allele. The $su(f)$ alleles that were used for this experiment were, $su(f)^+$, $su(f)^1$, $su(f)^94$, and $su(f)^{ls678}$. Each of these $su(f)$ alleles was recombined with each of the $lz$ alleles ($lz^I$, $lz^{34}$, $lz^{36}$, and $lz^{37}$). A total of sixteen different chromosomes were constructed. The virgin sources were the $su(f)$ stocks while the male sources were the $lz$ stocks. The general scheme for making these recombinants was as follows: $y^2 w^a f^I su(f) / y^2 w^a f^I su(f) X lz / Y$. Heterozygous virgin females ($y^2 w^a f^I su(f) / lz$) were selected, and mated to $+/Y$ males. The particular recombinant class that was selected was based on a crossover between $lz$ and $f$. The $w^a$ marker is located at map distance 1.5, the $lz$ marker at 27.7, and the $f$ marker at 56.7. Therefore the expected frequency of a single crossover between $lz$ and $f$ was 29% thus yielding a 14.5% frequency for the $lz f^I su(f)$ class of recombinant males. (However, in the cases involving the $lz^I$ allele, the single crossover desired was between $lz$ (27.7)

and v (33.0). The single crossover frequency is 5.3% thus yielding a 2.65% frequency for the lz f su(f) class of recombinant males.) The male progeny were screened for recombinants. A single recombinant male was isolated from each cross, and was mated to y w f. A description of these recombinant males, as well as the discussion of the effect of the various su(f) alleles on the various lz alleles is presented in the RESULTS section.

Tests on su(f) alleles

Three new mutations of su(f) were isolated, su(f)94, su(f)100, and su(f)R617. Several small recombination experiments were done in order to establish whether the newly isolated mutations were alleles of su(f). The new mutations, su(f)94 and su(f)100, were isolated in the EMS mutagenesis screen discussed above. The results of the mutagenesis will be further discussed in the RESULTS section. Both mutation have an extremely enhanced eye phenotype, i.e., very white eyes. The bristles, however, show very little forked suppression. The first set of tests was conducted only with the su(f)94 mutation since they were so much alike. The first step was to mate homozygous y2 wa f1 su(f)94 virgin females to wild type (+/Y) males. The wa allele on the y2 wa f1 su(f)94 chromosome is now referred to as wa* due to the possibility of the mutational lesion being at white. Heterozygous virgin females (y2 wa* f1 su(f)94 / +) were collected in the next generation and again mated to wild type (+/Y) males. Recombined males were screened in the next generation, and the findings will be discussed in the RESULTS section.

The su(f)R617 mutation was isolated as a reversion of su(f)1. The eye
phenotype is very similar to $su(f)^I$, that is, shows enhancement of $wa$, but the bristles are $forked$, that is not suppressed. The following cross was designed to test the question of whether these phenotypes are due to a new mutation at $forked$ or at $suppressor$ of $forked$. Virgin females homozygous for $y^2$ $wa$ $f^I$ $su(f)R617$ were mated to $y^2$ $wa$ $f^I$ $\cdot$ $y^+$ / $Y$ males. The $forked$ allele on the $y^2$ $wa$ $f^I$ $su(f)R617$ chromosome is now referred to as $f^I*$ due to the possibility of the mutational lesion being at $forked$. In the next generation heterozygous virgin females ($y^2$ $wa$ $f^I*$ $su(f)R617$ / $y^2$ $wa$ $f^I$ $\cdot$ $y^+$ ) were isolated and mated to wild type (+ / $Y$) males. Recombined males were screened in the next generation, and the findings will be discussed in the RESULTS section.

**In situ hybridization to polytene chromosomes**

The purpose of hybridizing the cloned $su(f)$ DNA to polytene chromosome squashes was to confirm its origin by localization to the correct bands. Chromosome preparations from the salivary glands of third instar larvae were made following the procedure described by Engels et al. (1986) with a few modifications. The larvae were grown in conditions described in the "Culture Techniques" section except that the parents were subcultured every day for four days. The parents remained in the bottle at 25°C for 1 day then were subcultured. The bottle remained an additional day at 25°C and was then transferred to 21°C. Larvae were collected at the late 3rd instar stage. The salivary glands were isolated in 0.75% NaCl then fixed in a drop of 45% acetic acid for 10-15 seconds. The glands were then transferred to a small drop of "1:2:3 solution" on an ethanol rinsed,
lenspaper wiped, siliconized coverslip for 5 minutes (min.). The cells were then "squashed" by sliding the coverslip onto an ethanol rinsed, lenspaper wiped, subbed slide. The cells were further squashed by gentle tapping. The polytene chromosomes were spread by a combination of tapping and pressing, and were flattened by being put under weights for at least 12 hours. The slide was dipped in liquid nitrogen for approximately 10 seconds, then the coverslip was removed by sliding a single edged razor under the corner and gently popping the coverslip off. The slide was rinsed in 95% ethanol twice for 5 min. then allowed to air-dry. The slide was reexamined for chromosome "flatness" and then stored at 4°C. The slides were treated in 2X SSC (made from 20X stock, 175.28 g NaCl, and 88.28 g NaCitrate per liter, pH7.0) at 65°C for 30 min. Two tanks of 70% ethanol and one of 95% ethanol were also heated to 65°C then removed to room temperature (RT) when the SSC incubation was complete. The slides were dehydrated by rinsing twice in 70% ethanol for 10 min., and once for 10 min. in 95% ethanol. After air-drying, the slides were stored at 4°C.

The su(f) DNA to be used as probes to the salivary chromosomes was labeled with digoxigenin-dUTP, a nonradioactive label as described in a short, technical report by de Frutos et al., 1989. The solutions for labelling were contained in the Genuis™ kit purchased from Boehringer Mannheim. The probe DNA was prepared, as described later in Materials and Methods, denatured at 95°C for 10 min., and immediately chilled on ice. To 1 ug of the denatured DNA was added 2 microliters (ul) of hexanucleotide mixture, and 2 ul dNTP labeling mixture. The mixture was then brought to a volume of 19 ul with sterile H2O followed by the
addition of 1 ul of Klenow enzyme. The mixture was incubated at 37°C for 12 hours. After the incubation, the reaction was stopped by adding 2 ul of EDTA (0.2 mol/L pH 8.0). The DNA was precipitated by adding 2 ul LiCl (4 mol/L), 50 ug tRNA, and 60 ul prechilled 100% ethanol and placing at -20°C for at least 2 hours. The DNA was centrifuged for 5 min. rinsed with cold 70% ethanol, centrifuged for 5 min., dried under vacuum, and resuspended in 50 ul TE (Tris-HCl, 0.1 mol/L, EDTA 0.01 mol/L, pH 8.0). The labelled DNA concentration was measured on the fluorimeter. The labeled DNA was then stored at 4°C.

The preparation for hybridization of the labelled probe DNA to the salivary chromosomes began with denaturation. The slides were incubated for 2 min. in 7% NaOH and rinsed 3 times for 5 min. each in 2X SSC. The slides were dehydrated by rinsing twice in 70% ethanol for 5 min. and once for 5 min. in 95% ethanol then air dried. The hybridization solution (5X SSC, 50% Formamide, 5% blocking reagent, 0.5% N-laureyl sarcosine, and 0.1% SDS) was prepared at least one hour in advance at 65°C. The probe DNA was brought to a concentration of 8 ng/ul then denatured at 95°C for 10 min. and immediately chilled on ice. One ul of denatured DNA was added to 5 ul of hybridization solution for each slide hybridized. The resulting 6 ul were placed on a clean, plastic coverslip, and picked up with the slide. The air bubbles were tapped out and the coverslip sealed to the slide with rubber cement. The hybridization reaction took place in a moist sealed chamber at 42°C for 16 hours.

Once the hybridization was complete, antibody binding and color development completed the process. The slides were rinsed twice in 2X
SSC at 42°C for 10 min. each. The slides were rinsed for 1 min. in buffer 1 (Tris-HCl, 0.1 mol/L, NaCl, 0.15 mol/L, pH7.5) then incubated in the antibody solution (1 ul of antibody conjugate to 5 ml buffer 1) at RT for 30 min. The slides were rinsed twice for 15 min. each in buffer 1 then 2 min. in buffer 3 (Tris-HCl, 0.1 mol/L, NaCl, 0.1 mol/L, MgCl₂, 0.05 mol/L, pH9.5). The slides were then incubated in the color solution (45 ul NBT solution and 35 ul X-phosphate solution in 10 ml of buffer 3) at RT in the dark for 2 hours. The color reaction was stopped by rinsing the slides in TE, pH8.0, for 5 min. at RT. The slides were air-dried, then examined under phase contrast and photographs taken of the in situ hybridization of labelled DNA to polytene chromosomes. The findings are presented in the RESULTS section.

Molecular Genetic Experiments

Cloning of su(f)

A 16.5 kilobase (kb) fragment containing all or part of the su(f) locus was cloned into the EMBL4 vector and was designated λ252.512. It was isolated by P-element transposon tagging and was kindly provided by Dr. Kevin O'Hare. The lambda phage containing the vector plus clone DNA was propagated by a method of plate lysis adapted from a procedure from Thomas and Davis (1975). Sterile LB broth (10 g bacto tryptone, 5 g bacto yeast extract, 5 g NaCl, pH to 7.3, plus 10 ml 20% maltose and 10 ml of 1M MgCl₂ per liter) was inoculated with a single colony of E. coli host bacteria and incubated while shaking overnight (O/N) at 37°C. The
bacteria were pelleted and resuspended in 20 ml of sterile 0.01 M MgSO₄. Phage dilutions of 10 fold over a 10⁸ range were prepared in SM (5.8 g NaCl, 2 g MgSO₄·7H₂O, 50 ml 1M Tris-HCl pH 7.5, 5 ml 2% gelatin, and 750 ml H₂O). 200 ul of cells were mixed with 100 ul of each phage dilution and incubated at 37°C for 30 min. The mixture was added to a 3 ml soft agar overlay and spread evenly onto LB plates. The plates were incubated at 37°C O/N. The titer of phage was calculated and the concentration of phage/ml that completely lysed the plate was determined. From this dilution of phage, 100 ul was again mixed with 200 ul of cells (per plate to be lysed) and incubated for 30 min. at 37°C. The mixture was plated in the same way and allowed to incubate at 37°C for approximately 10 hours.

After the phage had lysed the bacterial cells, 3 ml of SM saturated with chloroform was added to each plate and incubated at 37°C while shaking for one hour. The SM containing phage was removed, the plate was rinsed with an additional 1 ml of SM, and all SM was centrifuged for 10 min. at 6000g. The supernatant was removed and saved. A small aliquot was used to retiter the phage. The phage were then purified by pelleting in a swinging bucket rotor at 8K O/N. The pellet was resuspended in 800 ul of λdil (1 ml MgSO₄, 10 ml Tris-HCl pH 8.0, 0.2 ml 0.5M Na₂EDTA pH 8.0 per liter) and placed on ice for 15 min. The resuspended phage was spun in the microfuge to remove any remaining debris. The phage particles were further purified through two CsCl step gradients. Each step gradient was spun in the ultracentrifuge at 22.5K at 15°C for 2 hours. The phage layer was removed and stored at 4°C. DNA was isolated from 100 ul of
phage in CsCl by the addition of 10ul of 0.2 M EDTA, 2M Tris-HCl pH8.0, and 100 ul formamide. It was mixed and incubated at RT for 1 hour. The DNA was pelleted in a microfuge for 5 min. The pellet was rinsed with 70% ethanol, dried under vacuum, and resuspended in 100 ul TE. The DNA concentration was determined on the fluorimeter, after which it was restricted with various restriction enzymes and tested on a 1% agarose gel in TBE buffer (1.08g Tris, 5.5 g Boric Acid, 4 ml 0.5M Na2EDTA pH8.0 per liter).

Subcloning of λ252.512

The λ252.512 clone was subcloned to smaller fragments inserted into plasmid vectors for several reasons. Plasmid vectors maintained in host bacterial strains are much easier to propagate and DNA isolation is simple and rapid, unlike the phage vectors. Also, fragments in the size range of 2-6 kb are convenient sizes for use as probes. Subclones of λ252.512 were made following the DNA isolation described above. The vector plus 16.5 kb insert DNA was restricted with either EcoRI or XhoI in order to generate different size fragments. Restricted DNA fragments were mixed with the appropriately restricted pIBI20 DNA at a ratio of approx. 1:5. This DNA mix was ethanol precipitated then resuspended in 11 ul sterile H2O and then incubated at 65°C for 5 min. T4 DNA ligase, 1 ul, and 3 ul of 5X ligation buffer were added and the DNA was ligated O/N at 15°C. The ligation reaction was stopped by incubation at 65°C for 5 min. Bacterial cells from the E.coli strain, JM83, were made competent according to the procedure from Maniatis et al. (1982). A 3 ul aliquot of
ligated DNA was diluted to 100 ul with TE and added to 200 ul of competant cells, then placed on ice for 30 min. The mixture was heat shocked for 2 min. at 42°C. One ml of LB was added to the mixture and incubated at 37°C for 30 min. Varying volumes of the mixture were plated onto LB plates containing X-gal and ampicillin and incubated O/N at 37°C.

Selection for bacterial colonies carrying plasmids containing inserts is based on an antibiotic resistance and a color production scheme. Since the plasmid carries the only copy of the ampicillin resistance gene, the bacteria that do not carry a plasmid do not survive and do not form colonies. When X-gal is present in the media it combines with the protein product transcribed from the lac Z gene on the pIBI20 plasmid to give a blue color to the bacterial colony containing plasmid without insert. The single restriction site for both EcoRI and XhoI were located within the lacZ gene of the plasmid. An interruption of this gene, i.e., an insertion of foreign DNA would result in the bacterial colony losing the ability to produce a blue color in the presence of X-gal. Therefore, colonies carrying plasmids containing insert DNA are white in color provide simple selection. White bacterial colonies were streaked onto LB plates with X-gal and ampicillin for a second selection. The plasmid DNA was isolated from many different white colonies according to the alkaline lysis mini preparation method (Maniatis et al., 1982). The DNA was restricted with the appropriate restriction enzyme and analyzed on a 0.7% agarose gel in TBE. Plasmids identified as pR-1-2 (containing a 3.2kb EcoRI fragment), pR-9-42 (containing a 5.3kb EcoRI fragment), pX-9-2 (containing a 4.4kb XhoI fragment), and pX-3-3 (containing a 2.7 kb XhoI fragment) were
detected and the bacterial stocks amplified. The subcloning is diagrammed in Figure 1. The subclones were confirmed to contain the fragments isolated from λ252.512 DNA by Southern hybridization (procedure described later). The subclones are maintained in the *E. coli* strain, JM83 and are stored in glycerol stocks.

*Drosophila* DNA isolation

Genomic DNA from *Drosophila* was isolated in order to analyze many different alleles of *su(f)* by probing with the subcloned fragments discussed above. However, before the description of how the *su(f)* alleles were analyzed, the method (based on protocols by Jowett, 1986) for isolating genomic DNA from *Drosophila* is presented. DNA was isolated from adult *Drosophila* females heterozygous for the *su(f)* allele of interest and FM7. The DNA was isolated by collecting at least 200 etherized flies in an 50 ml centrifuge tube and quickly freezing them with liquid nitrogen. They were transferred to a cold tissue grinder and 2 ml of lysis solution (2X lysis buffer, 10% SDS, 15mM spermine, 50 mM spermidine and sterile water to 10 ml) was added. The tissue was homogenized and poured into the centrifuge tube. The tissue grinder was then rinsed with another ml of lysis solution, which was added to the homogenized tissue. A volume of 30 ul of 10mg/ml of proteinase K was added to the homogenate. The tube was then covered with parafilm and incubated at 37C while slowly shaking for 2 hours.

The next step was DNA extraction. This was done by extracting once with phenol, followed by 2 phenol / chloroform / isoamyl alcohol (25:25:1)
extractions, and one chloroform / isoamyl alcohol extraction. The aqueous layer was removed after the final extraction and 1/2 volume of 7.5M ammonium acetate and 3 volumes of cold 100% ethanol were added. The DNA was precipitated O/N at -20°C. The DNA was centrifuged at 8000g for 10 min, the ethanol was carefully removed, the tube was covered with parafilm with a few holes poked through, and the DNA pellet was dried in a vacuum desicccator for 1 hour. The pellet was resuspended in 400 ul of TE and transferred to an eppendorf tube. RNase (DNase free) was added to a final concentration of 100 ug/ml and incubated at 37°C for 30 min. The DNA was again extracted with phenol / chloroform / isoamyl alcohol followed by an extraction with chloroform/isoamyl alcohol. The aqueous layer was removed and 2 volumes of water saturated ether was added. The tube was inverted once, then opened and spun for 2 min. under vacuum to remove the ether layer. The aqueous layer containing DNA was incubated at RT for approximately 10 min., then 3 volumes of cold 100% ethanol was added and the DNA precipitated at least 30 min. at -20°C. The DNA was pelleted in a microfuge, rinsed with 70% ethanol, dried under vacuum, then resuspended in 200 ul TE usually at 4°C O/N. The concentration of DNA was determined with the fluorimeter, followed by restriction of 2 ug, and separation on a 1% agarose gel in TBE.

**Genomic Southern hybridizations**

By using the Southern Hybridization technique, as originally described by E. M. Southern (1975), of separating genomic DNA on agarose gels and transferring the DNA onto membranes, many different $su(f)$ alleles were
analyzed by probing with the subcloned fragments discussed above. The
objective was to detect restriction fragment length polymorphisms (RFLPs)
among the su(f) alleles, and hopefully relate molecular lesion to mutant
phenotype. For each strain of su(f), 2 ug of DNA were restricted in a
reaction mixture of 2 ul of the particular enzyme, 2 ul of the appropriate
10X restriction buffer, and sterile H2O to 20 ul at 37°C for 1 hour. The
DNA was separated on a 0.7% 20 X 25 cm agarose gel at 50V for 18 hr in
TBE. The gel was stained in TBE containing ethidium bromide (200
ng/ml) and photographed using Polaroid™ Type 55 film. The gel was
trimmed to a 20 X 20 cm size by cutting just below the wells. The gel was
then depurinized for 30 min in a solution of 0.25 M HCl, denatured for 1
hour in a solution of 0.5 M NaOH and 1.5 M NaCl, and neutralized for 1
hour in a solution of 0.5 M Tris-HCl, pH5.4 in 20X SSC all while gently
shaking at RT. Double thickness Whatman™ paper, measuring about 30
cm², was placed on several layers of Saran Wrap™ and saturated with 20X
SSC. The gel was then placed on the SSC soaked paper and a sheet of
precut BRL™ nitrocellulose membrane, presoaked in 2X SSC was placed
on the gel. After all air bubbles were removed, the edges of the Saran
Wrap™ were brought to the edges of the gel to enclose the buffer and
allow it to only transfer through the gel. Two sheets of 20 X 20 cm dry
Whatman paper were placed on the membrane and an approximately 3 inch
stack of 20 X 20 cm size absorbant filter paper was placed on top. A glass
plate was placed on the filter paper and weights were placed evenly on the
glass plate. The DNA was allowed to transfer from gel to membrane
overnight. The DNA transfer is achieved due to the capillary action of the buffer.

Following the transfer the apparatus was disassembled and the membrane was soaked in 6X SSC for 5 min. The membrane was then air-dried for about 15 min and then baked under vacuum at 70°C for 2 hours. The membrane was then sealed in plastic and 60 ml of prehybridization solution (15 ml 20X SSC, 6 ml 50X Denhardt's, 1.2 ml 1 M Na(H)PO4 pH 6.5, 30 ml deionized formamide, 3 ml denatured salmon sperm DNA at 5 mg/ml, and 4.8 ml sterile H2O) was added. The membrane was incubated in the prehybridization solution for at least 2 hours at 42°C while shaking. The next step in this procedure was the hybridization and preparation for autoradiography. These steps are discussed below following the description of isolation of DNA for probes and the labelling technique.

**Isolation of probe DNA, labelling, and hybridization**

In the RFLP analyses of *su(f)* alleles, several different subcloned DNA fragments of 1252.512 were used as probes. Each probe was isolated, labelled, and hybridized to the genomic Southern blots. The first probes used were simply the isolated plasmid DNA of each subclone. The DNA was restricted by the same enzyme used to subclone that particular fragment. The DNA was then denatured and labeled according to the procedure accompanying the Boehringer Mannheim Random Primed DNA Labeling Kit using 32P labelled dCTP as the labelled nucleotide. At a later point in the process of making probes for the Southerns, a different method was used to isolate probe DNA. Plasmid DNA was still isolated as
previously described, but the subcloned insert was restricted and separated electrophoretically from the vector DNA on a 1% low EEO agarose (Seakem GTG™) gel in TAE (4.84g Tris, 1.14 ml glacial acetic acid, and 2 ml 0.5M EDTA pH8.0 per liter). The subcloned fragment was then cut out of the agarose. The DNA was isolated from the agarose using the reagents and protocol of the Bio101 GENECLEAN™ kit. This procedure allowed for the isolation of insert only DNA for use as probes. Recovery of pure, insert only DNA was approximately 30%. This DNA was also denatured and labelled according to the procedure accompanying the Boehringer Mannheim Random Primed DNA Labeling Kit using 32P labelled dCTP as the labelled nucleotide. This DNA was superior for use as probes. Proportionally more unique \textit{su(f)} was labelled due to the fact that insert only DNA was being labelled instead of the entire vector plus insert. For both labelling procedures, the unincorporated nucleotides were removed by using a G-50 sephadex spin column.

The labelled, freshly denatured probe DNA (25-50 ng) was added to 20 ml of hybridization solution (5 ml 20X SSC, 400 ul 50X Denhardts, 400 ul 1M Na(H)PO4 pH 6.5, 10 ml deionized formamide, 800 ul denatured salmon sperm DNA at 5 mg/ml, and 3.4 ml sterile H2O). Prehybridization solution was removed from the bag containing the membrane and replaced with probe plus hybridization solution. The hybridization reaction was allowed to incubate at 42°C while shaking O/N. The solution was then removed and the membrane was washed. The first three washes were for 10 min each at RT while shaking using 300 ml of 2X SSC and 0.1% SDS. The next two washes were for 30 min each at 55°C while shaking using
300 ml of 0.1X SSC and 0.1% SDS. The membranes were air-dried for 5 min then wrapped in Saran Wrap™. The membrane was the secured in an X-ray cassette and, while in the dark, X-ray film placed on top. The cassette was sealed, covered tightly with aluminum foil, then placed at -70°C for 3-4 days. Following exposure, the cassette was thawed at room temperature, and the film developed. A second exposure was often set up which took an additional 4-8 days to expose. The autoradiograms were dried and examined to determine if any RFLPs were present.
RESULTS AND DISCUSSION

Genetic Experiments

EMS mutagenesis

Approximately 100 potential mutants were recovered from the G1 EMS mutagenesis screen as described in the Materials and Methods. These potential mutants were selected because they showed the enhanced \( w^a \) eye phenotype and/or the suppressed bristle phenotype. Each of the 100 individual potential mutants were further tested in G2 screen by mating sibling females containing the mutagenized chromosome \( y^2 \; w^a \; f^1 \; / \; FM7 \) to \( y^2 \; w^a \; c^6 \; f^1 \; DfVE738 \; / \; B^S Y \) males. True \( su(f) \) mutations would result in female progeny, of the genotype \( y^2 \; w^a \; f^1 \* \; / \; y^2 \; w^a \; c^6 \; f^1 \; DfVE738 \), showing a visible \( su(f) \) phenotype. Only two of the 100 potential mutants appeared to be true \( su(f) \) mutants. They were isolated as number 94 and 100 in the G1 screen and were therefore designated as \( su(f)^{94} \) and \( su(f)^{100} \), respectively. They were isolated from bottles that contained the same 10 mutagenized male parents. Both of these mutants appeared \( w^a \) enhanced but showed very little \( f \) suppression. They were put into stock by mating \( y^2 \; w^a \; f^1 \; su(f)^{94} \; / \; FM7 \) or \( y^2 \; w^a \; f^1 \; su(f)^{100} \; / \; FM7 \) to \( FM7 \; / \; Y \) males. These \( su(f) \) mutants were not further analyzed until they were subjected to restriction fragment polymorphism analysis which is discussed in the Molecular Experiments Results Section. Due to these findings, these mutant stocks were then subjected to extensive genetic testing which is discussed later in this section.
**TEM and DEB mutagenesis**

In the initial attempt at TEM mutagenesis, the mutagenized males were mated and subcultured as described in the Materials and Methods Section. A total of 32 bottles were set up at 21°C, the parents cleared after 8 days, and on day 17 the G1 generation was screened. However, in all of the bottles very few eggs were laid and, consequently, even fewer progeny emerged. Therefore the screening was impossible due to the extremely low number of flies. The possible causes were analyzed and the conclusion was drawn that the TEM must have been improperly weighed thus leading to an exceedingly high concentration of mutagen. TEM is a very lightweight powder and difficult to weigh. Consequently, obtaining an accurate and reliable concentration of the mutagen in solution can be difficult (T. Grigliatti, University of British Columbia, Vancouver, British Columbia, Canada, personal communication). Therefore in the next set of mutagenesis experiments concentrations of TEM were varied in order to establish the optimal concentration for TEM usage. Two sets of mutagenesis experiments were completed where the concentrations of mutagen were varied. As discussed in the materials and Methods Section, TEM is a powder and is made up in a sterile 1% sucrose solution to a final concentration of 0.15mM. A final concentration of 0.15mM is equivalent to 1X concentration. In the first, the following concentrations of TEM were tested: 2X concentration, 1X concentration, 0.5X concentration, and 0.25X concentration. A control using untreated males was also scored. A summary of the numbers of males and treated females that were scored is
presented in Table A-1, (in Appendix A). The number of treated chromosomes screened for this experiment was 1751. Potentials mutants were picked up in the G1 screen and were analyzed together with those potentials isolated in the second round of TEM mutagenesis.

The second set of concentrations tested in the TEM mutagenesis were: 1X concentration, 0.8X concentration, 0.6X concentration, 0.4X concentration, and 0.2X concentration, as well as untreated males scored as the control. A summary of the numbers of males and treated females that were scored in the second experiment is presented in Table A-2, (in Appendix A). The number of treated chromosomes screened for this experiment was 2527 for a total of 4278 for both the 1st and 2nd experiments.

Each of eleven potential mutants isolated in either the 1st or 2nd round of TEM mutagenesis was mated to $y^2 w^a c^6 f^l DfVE738 / B^sY$. The G2 progeny were counted and the results recorded in Table A-3 located in Appendix A. It was expected that if a deletion at su(f) occurred, then the $y^2 w^a f^l * \cdot y^+ / y^2 w^a c^6 f^l DfVE738$ females should be lethal, or at least exhibit a strong su(f) phenotype. The sibling males carrying the treated chromosome would have been used to establish a stock. In the case of potentials #1 and #5, these males were further tested in the G3 generation due to low numbers of progeny in the G2. In every one of the eleven cases the females carrying the treated chromosome were not lethal nor did they exhibit the su(f) phenotype, consequently eliminating them as potential su(f) mutants. Even the 3rd generation testing in the cases of #1
and #5 did not generate females exhibiting either the su(f) phenotype or lethality.

Another set of mutagenic experiments employed the new mutagen, diepoxybutane, DEB, as described earlier. This is a mutagenesis method of obtaining potential intragenic deletions as described by Olsen and Green, 1982. Deletions are mutational lesions that can be detected at the molecular level, much more readily than mutational lesions that are caused by point mutations. The G1 progeny were not counted by hand in this experiment since a much larger screen was completed. The experimental bottles were screened beginning on day 17 and for each day following for eight days. Potential mutants were isolated, then further characterized. All remaining progeny, both males and females were added to alcohol, water, and soap morgue. From the control cross, in which males were not treated with DEB, males and females were separated, counted, and added to two small morgues. The results of the "No DEB" control cross are presented in Table A-4, located in Appendix A. The "Cross 1" and "Cross 2" results are because DEB was administered on two different occasions and a control done each time. In order to "count" the G1 progeny, it was necessary to have a counted group of both females and males in separate morgues since only G1 females carried the treated chromosome. The control crosses produced 641 females and 645 males. To have a total of 1300 females, 659 females were counted from some of the experimental bottles and added to the female small morgue. For the same reason, 655 males were counted from experimental bottles and added to the male small morgue for a total of 1300 males. After all of the bottles were screened the flies in the three
morgues were washed, dried, and weighed. Following are the calculations to determine the number of treated chromosomes screened:

Control females = 1300 females = 0.3245 g  
Control males = 1300 males = 0.2176 g  
Total weight for controls = 0.5421 g

Experimental morgue weight = 5.9497 g

% of males and females
0.2176 g / 0.5421 g = 40.14%  0.3245 g / 0.5421 g = 59.86%
males by weight  females by weight

# of females (treated chromosomes) screened
5.9497 g (total weight) \times 0.5986 (female \%) = 3.56 g (total female weight)
therefore:

0.3245 g \times 1300 females = 3.56 g \times females \times 14,262 females

screened

# of males screened
5.9497 g (total weight) \times 0.4014 (male \%) = 2.38 g (total male weight)
therefore:

$$0.2176 \text{ g} = 1300 \text{ males}$$
$$2.38 \text{ g} \times \text{ males} \times X = 14,278 \text{ males screened}$$

**TOTAL = 28,540 males and females**

Another control was conducted in order to determine the number of lethal mutational lesions on the X chromosome caused by the mutagen. Treated males were mated to \(yw^{f}f\) females. The expected F1 progeny were \(y^{2}w^{d}Y^{d}f^{d} \times Y^{d}f^{d} / Y \) males, \(yw^{f}f\) attached X females, and very few triplo-X females. Since the male has only one X chromosome, any deletions which result in recessive lethality will result in lethality for the male. Therefore a standard rule to determine the potency of the mutagen is to recover males in a ratio of 50% to the number of females recovered. In Table A-5 (Appendix A) the results from this control cross are listed. The "Cross 1" and "Cross 2" results are because DEB was administered on two different occasions and a control done each time. In Cross 1 the male / female ratio is 99.3% which is very poor compared to the expected 50%. In Cross 2 the ratio is 80.7% for an average of 88.1%. As would be predicted, 7 of the potentials came from experimental Cross 1 and 22 came from Cross 2. These results, coupled with the fact that no new DEB induced mutations were obtained indicates that the concentration strength of the DEB mutagen solution needs to be greatly increased if this would be a potential method of obtaining intragenic deletions (Olsen and Green, 1982).
Potential mutants were isolated and mated as described in the F2 Cross for the TEM mutagenesis. In some cases as noted in the "origin" column of Table A-6 (Appendix A), the potential females were nonvirgin. In these cases, they were mated to $y^2 w^a cf^6 f^I DfVE738 / B^s Y$ males, but some of their expected progeny were of the genotypes listed in the last four columns of Table A-6. In the cases where a virgin female was isolated as a potential mutant, these genotypes were not expected and thus an X was placed in the appropriate columns. When the potential mutant females were nonvirgins, their female progeny were scored and listed in parenthesis due to the inability to distinguish between phenotypes of two genotypes. For example a $y^2 w^a f^I * * y^+ / y^2 w^a cf^6 f^I DfVE738$ female which is a nonmutant will have apricot eyes and forked bristles as will the nonmutant $y^2 w^a f^I * * y^+ / y^2 w^a f^I su(f)^I$ female. This is also true for the $y^2 w^a f^I su(f)^I / y^2 w^a cf^6 f^I DfVE738$ which will appear $w^a$ enhanced and $f$ suppressed (the $su(f)^I$ phenotype) and will look exactly like the $y^2 w^a f^I su(f)^I / y^2 w^a f^I su(f)^I$ female. In the case of Potential #30, a male was derived from a test of the Potential #1 cross. Its genotype was $y^2 w^a f^I * * y^+ / Y$ and was white eyed ($w^a$ enhanced) but not $f$ suppressed. It was mated to $yw^f$ and several males were generated. Some of these males were mated to $y^2 w^a f^I su(f)^I / y^2 w^a f^I su(f)^I$ virgin females in order to screen the $y^2 w^a f^I * * y^+ / y^2 w^a f^I su(f)^I$ genotype and some were mated to $y^2 w^a cf^6 f^I DfVE738 / FM7$ virgin females to test $y^2 w^a f^I * * y^+ / y^2 w^a cf^6 f^I DfVE738$ genotype. Therefore all results from the test crosses of the potentials are listed in Table A-6. In every one of the 30 cases except 1, females of the $y^2 w^a f^I * * y^+ / y^2 w^a cf^6 f^I DfVE738$
genotype were not lethal nor did they exhibit the su(f) phenotype, consequently eliminating them as potential su(f) mutants. In one case, #14, neither the $y^2 w^{a} f^l \times y^+/y^2 w^{a} c^{5} f^l DfVE738$ females were present, nor their siblings, $y^2 w^{a} f^l \times y^+/B^s Y$ males. In this case, a deletion probably occurred on the treated chromosome within the region of the VE738 deficiency (accounting for the lethality of the female class), but outside the region covered by the $B^s$ duplication (accounting for the lethality of the male class). Therefore the deletion was confirmed to be outside su(f).

The value of an intragenic deletion of su(f) has previously been established. The necessity of obtaining an intragenic deletion of su(f), which might result in a null mutation, is still present and DEB is still viable as a mutagen. However, in view of the fact that the results were negative from a cross that screened over 14,000 chromosomes, it would be necessary to make a few changes. The first and most important change to be made would be the concentration of mutagen to be used. Instead of testing various concentrations of mutagen using the experimental cross, as was done in the TEM experiment, it would be far better to test various concentrations of DEB using the cross of treated males mated to attached X females. This cross was used as a control in the DEB experiment to determine if a male/female ratio of 50% due to male lethality was being achieved. This same analysis can be used in a new DEB mutagenesis using this cross as a screen to establish the optimal concentration of mutagen needed to achieve the 50% lethality of males. After an optimal concentration of mutagen has been determined a very large scale screen can...
be started. The point "very large scale screen" is stressed due to results found in the literature. According to Olsen and Green in 1982 a screen of 94,000 chromosomes yielded 1 intragenic deletion of the white (w) locus and a second experiment of 55,000 chromosomes produced 2 similar deletions. Ten other positive w deletions were obtained but each deletion included other loci as well. Fewer deletion mutants were obtained for the y locus but the exact number was difficult to establish since they did not have a good, objective genetic test. After reviewing this reference, the need for a much larger screen became very evident; and even though the concentration of DEB they used was 0.005M, the same as the concentration used in the DEB mutagenesis of su(f), establishing the optimal concentration of mutagen, is important.

**Single P element mutagenesis**

The purpose of this mutagenesis is twofold. The first is to obtain a Pftz induced mutation of su(f). The insertion of the P element at the su(f) locus will allow for a more in-depth study of su(f), its developmental profile (where and when it is transcribed), and the cell type in which it is transcribed. The Pftz element should be transcribed when su(f) is transcribed and therefore would have enabled us to learn a great deal about su(f). The second reason is that once a Pftz su(f) mutant is obtained, the element can be induced to jump back out of the locus much the same as the original transposition. Often times, when these elements jump, they do so with imprecise excision, leaving behind a small deletion. Obtaining a small intragenic deletion of su(f) is also a major goal of this mutagenesis. Many
of the current su(f) alleles are believed to be caused by point mutations, which makes molecular detection of the lesion very difficult. Therefore, a group of intragenic deletions would not only make mapping at the molecular level easier, but they would also be beneficial in the developmental studies of su(f).

This work involved a relatively new technique of mutagenesis (Cooley et al., 1988a). The ability to induce hybrid dysgenesis with a single P element which has been crossed into the genome provides a very useful tool of mutagenesis. The largest part of this work involved construction of the chromosomes that were to be used. The general cross is outlined in Figure B-1 in Appendix B. The stocks containing the P(ftz- βgal)123.1 P element and the stock containing the ry^06 "wings clipped" (ry+) were kindly provided by Dr. Spyros Artavanis-Tsakonas. The presence of the Pftz element within the genome can be detected by a chemical test using X-gal to stain embryos 12-18 hrs. old. If the Pftz element was present at least portions of the embryo turned blue. Females heterozygous for the y^2 w^a f^1 chromosome and the P(ftz- βgal)123.1 chromosome were collected as virgins. They were mated to wild type males followed by selecting recombinant males. Since the presence of the P element is not phenotypically detectable, these males had to be mated to yw^f females in order to build the stock. Approximately 20 male lines were established. These parents were allowed to lay eggs for 8 hours, then were removed. The eggs were aged to 12-18 hours, at which time the chorion was removed. The eggs were treated in a series of solutions, the final of which contained X-gal. They were incubated overnight, and if any of the eggs
turned blue, it was assumed that the P element was present in the genome. Of 20 lines, 4 lines were positive for the blue color.

At this time, further confirmation of the presence of the P element was necessary. Salivary gland chromosomes were isolated from these 4 lines, designated as 1-4, 2-2, 2-7, and 2-8. Two probes were used for *in situ* hybridization to the salivary gland chromosomes. The first was the entire linearized plasmid clone, P2 25.1, which contains an entire P element cloned from the 17C region of the genome (O'Hare and Rubin, 1983) as discussed in the Materials and Methods. The second probe was a 0.84 kb Hind III fragment containing an LTR of the P element isolated from this plasmid clone. Both were labelled using the nonradioactive labelling Genuis™ kit as described in Materials and Methods. Both probes gave the same result: all strains showed multiple sites of hybridization, both on the X chromosome and on the autosomes. All strains showed at least six sites of hybridization on the X chromosome, and a total of 25-30 sites throughout the genome. None of the hybridization sites corresponds to the site of PI23.1 in the control. Three separate hybridization experiments were done on each strain, all of which gave identical results. The stringency of hybridization was varied (from low to high) by altering the temperature of hybridization and washing (from 32°C to 48°C). This had no effect on the hybridization pattern, confirming that the observed bands are not the result of nonspecific hybridization. In each experiment a control (PI23.1) set of chromosomes was included. These showed a single hybridization site with the 0.84 kb Hind III fragment probe, in the normal PI23.1 location, at approximately 4B-D, and a second site at the white
locus with the intact π2 25.1 plasmid probe (J. Girton, Department of Genetics, Iowa State University, personal communication). The latter is not expected. This second site was not observed in a second series of control hybridizations. However a site of hybridization was localized to approximately 17 C in this second series, corresponding to hybridization of the genomic DNA in the plasmid probe (M. Gorman, Iowa State University, personal communication). The hybridization to the white locus in the P123.1 control suggests that this strain contains a highly active element, capable of transposing even in a nondysgenic cross (P. Bingham, State University of New York, personal communication).

These results suggest that (1) the P123.1 element was mobilized over several generations in the crosses used to construct the chromosomes necessary for mutagenesis. (2) This mobilization may have included duplication / replication of the element. (3) It is also possible that there are sequences in these stocks that are homologous to the terminal repeat of a P element. The stocks were tested for P element activity by crossing to an indicator sn^W stock and were shown to have no P element activity. Thus any P element sequences that are present must consist of deleted, nonfunctional sequences. In light of these results, it was decided not to continue this single P element mutagenesis until the reason for multiple P element hybridizations was determined.

Complementation studies

Allelism has been established for many su(f) mutations through complementation studies (J. Girton, personal communication). The results
of these experiments (as discussed in the Introduction) provided the foundation and hypotheses for this complementation study. In order to determine whether several new \textit{su(f)} mutations, discussed above, were actually alleles, a series of complementation studies was undertaken. The known \textit{su(f)} alleles, \textit{su(f)}$^1$, \textit{su(f)}$^+$, \textit{su(f)}$^{ts726}$, \textit{su(f)}$^{ts678}$, and a \textit{su(f)} deficiency, \textit{VE738}, were compared to the \textit{su(f)} mutations, \textit{su(f)}$^{94}$, \textit{su(f)}$^{100}$, and \textit{su(f)}$^{R617}$. They were tested for allelism by crossing \textit{inter se} at three different temperature regimes, as discussed in the Material and Methods Section.

The mutations \textit{su(f)}$^{94}$ and \textit{su(f)}$^{100}$ were selected for this study for two reasons. The first was due to the recent finding of a Restriction Fragment Length Polymorphism (RFLP) associated with these mutations. The RFLP for both mutations is thoroughly discussed in the Molecular Results Section. One of the most interesting findings was that both mutations showed the exact same RFLP. It was upon the analysis of these molecular results that an hypothesis about the origin of these mutants became necessary. As discussed in the EMS Mutagenesis Results Section, these mutations were isolated from bottles that contained the same 10 mutagenized male parents. Since these mutations showed the same RFLP and they were isolated from the same group of mutagenized parents, they may in fact be due to a premeiotic mutagenic event in a single male parent. Only this occurrence would explain how two mutants, isolated in separate vials in a G2 screen, could show the exact same molecular lesion. The second reason was the interesting homozygous phenotype for both of these mutations. Females heterozygous for the mutagenized chromosome over
the deficiency chromosome show moderate $w^a$ enhancement and moderate $f$ suppression. Following the original mutagenesis, the mutagenized chromosomes were never examined as homozygous individuals. The stocks of $y^2 w^a f^1 su(f)^{94} / FM7$ or $y^2 w^a f^1 su(f)^{100} / FM7$ females $X FM7 / Y$ males were maintained for many generations. At a later date, when thorough documentation of these mutant phenotypes became a necessity, the homozygous $y^2 w^a f^1 su(f)^{94} / y^2 w^a f^1 su(f)^{94}$ and $y^2 w^a f^1 su(f)^{100} / y^2 w^a f^1 su(f)^{100}$ females were examined. Both strains of homozygous females appeared to show extreme $w^a$ enhancement, that is, the eyes were almost completely white, and less $f$ suppression. At this point, it was important to know if this homozygous phenotype was a result of a true mutation at $su(f)$ and if the $su(f)^{94}$ and $su(f)^{100}$ mutations were alleles.

The mutation $su(f)^{R617}$ was included in both the molecular analyses and this complementation study. It was isolated as a reversion of $su(f)^I$. Its homozygous phenotype showed extreme $w^a$ enhancement, just like $su(f)^I$, but very little $f$ suppression, unlike $su(f)^I$. Due to the homozygous phenotype that is similar to the $su(f)^{94}$ and $su(f)^{100}$ mutations, the $su(f)^{R617}$ mutation was included in this complementation study.

The first step of this study was to document the homozygous phenotype for each of these alleles and mutations. As discussed in the Materials and Methods Section, the homozygous females for the above $su(f)$ alleles and $su(f)$ mutations, were counted and scored for $w^a$ enhancement and $f$ suppression. The results of this scoring are presented in Tables 3 and 4. On the correct day (day 13 for 29°C, day 16 for 25°C, and day 21 for 21°C) the progeny were etherized and the homozygous females were
counted. This number is found in the row labelled "# females" in both Tables 3 and 4. From these females, 10 nonvirgins were set aside for further scoring. The first analysis made was the level of \( w^d \) enhancement. Each female was assigned a numerical value reflecting the amount of color in the eye. The scale ranged from 1 to 5. No enhancement, showing phenotypically \( w^d \) eyes was a score of 1. Complete enhancement, phenotypically white eyes, was a score of 5. All phenotypes between the 1 and 5 maximums, and were assigned a corresponding score relative to its phenotype. This numerical assignment was based on the comparison to two genotypes, that of \( y^2 w^d f^D su(f)^+ \) (\( w^d \) eye phenotype) and \( ywT \) (white eye phenotype) raised at 25°C (Figure 2 A and B). Each of the ten flies was given an "eye phenotype score", then the numbers were averaged. The resulting number is presented on the row labelled "eye color" in both Tables 3 and 4. The level of forked suppression was determined by scoring the number of bristles that were forked for 12 preselected dorsal thoracic and scutellar bristles. The bristles analyzed were the anterior and posterior scutellars, the anterior post-alar, the posterior supra-alar, and the anterior and posterior dorso-centrals. On each fly both medial and lateral sets of bristles were scored. Each bristle was determined to be "forked" or "not forked" and so the "forked phenotype score" for each fly ranged from 1 to 12. Each of the 10 flies were given a score and the numbers were averaged. The resulting number is presented in the row labelled "# forked" in both Tables 3 and 4. The definition of a "forked bristle" ranges from an extremely thickened and gnarled looking bristle to one which looks normal in thickness and length but has a slight bend at the tip or part
of the tip missing. Finally the columns labelled A and B for each
temperature regime represent two different trials. The two separate counts
and scoring are from one bottle for each trial.

The homozygous $y^2 w^a f^l $ su(f)$^+$ / $y^2 w^a f^l $ su(f)$^+$ female shows no $w^a$
enhancement and no forked suppression. In Table 3, it is shown that at all
three temperatures, the eye phenotype score is 1.0. All 12 bristles are
almost always forked as shown by the forked phenotype scores which range
from 11.4 to 12.0. Temperature does not seem to affect either level. As
stated earlier, the eye phenotype is seen in Figure 2 A while the bristle
phenotype for the homozygous su(f)$^+$ fly, also raised at 25°C, is shown in
Figure 2 D. The next phenotype analyzed was that of the $y^2 w^a f^l $ su(f)$^l$/
$y^2 w^a f^l $ su(f)$^l$ female. The eye phenotype of this female raised at 25°C is
shown in Figure 2 C, while the bristle phenotype is shown in Figure 2 E.
The level of $w^a$ enhancement varies with temperature. At 29°C, the eyes
are $w^a$ enhanced at a medium level, while the enhancement gets stronger at
25°C and strongest at 21°C. This finding is consistent with those obtained
in earlier su(f) complementation studies (J. Girton, personal
communication). These eye phenotypes at the other temperature regimes
are shown in Figure 3 A and B. Figure 3 A shows the phenotype of the
su(f)$^l$ allele raised at 29°C while Figure 3 B shows the phenotype of the
su(f)$^l$ allele raised at 21°C. The strong forked suppression, however,
seems to stay constant for each temperature regime. The "forked score"
ranges from 0.3 to 1.0 as shown in Table 3.

The homozygous su(f) mutations were analyzed next. Both the su(f)$^{94}$
and su(f)$^{100}$ mutations show very strong $w^a$ enhanced phenotype. For
Table 3. Results of the Complementation Study for Homozygous Genotypes

<table>
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<th>Genotype</th>
<th>Description</th>
<th>29 °C</th>
<th>25 °C</th>
<th>21 °C</th>
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<tr>
<td></td>
<td></td>
<td>A 13</td>
<td>B 13</td>
<td>A 16</td>
</tr>
<tr>
<td>su(f) + / su(f) +</td>
<td>eye color</td>
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<td>1.0</td>
<td>1.0</td>
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<tr>
<td></td>
<td># forked</td>
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<td>11.9</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td># females</td>
<td>51</td>
<td>9</td>
<td>86</td>
</tr>
<tr>
<td>su(f) 1 / su(f) 1</td>
<td>eye color</td>
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<td>2.4</td>
<td>3.8</td>
</tr>
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<td>1.0</td>
</tr>
<tr>
<td></td>
<td># females</td>
<td>22</td>
<td>10</td>
<td>103</td>
</tr>
<tr>
<td>su(f) 94 / su(f) 94</td>
<td>eye color</td>
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<td>4.9</td>
<td>4.8</td>
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<td>11.2</td>
</tr>
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<td></td>
<td># females</td>
<td>71</td>
<td>10</td>
<td>111</td>
</tr>
<tr>
<td>su(f) 100 / su(f) 100</td>
<td>eye color</td>
<td>4.5</td>
<td>5.0</td>
<td>4.8</td>
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<td>11.9</td>
</tr>
<tr>
<td></td>
<td># females</td>
<td>65</td>
<td>10</td>
<td>112</td>
</tr>
<tr>
<td>su(f)R617 /su(f)R617</td>
<td>eye color</td>
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<td>3.0</td>
<td>3.5</td>
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</tr>
<tr>
<td></td>
<td># females</td>
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<td>10</td>
<td>112</td>
</tr>
<tr>
<td>su(f) 726 / su(f) 726</td>
<td>eye color</td>
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<td>2.3</td>
<td>3.1</td>
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</tr>
<tr>
<td></td>
<td># females lethal</td>
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<td>96</td>
</tr>
<tr>
<td>su(f) 67g / su(f) 67g</td>
<td>eye color</td>
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<td>3.1</td>
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</tr>
<tr>
<td></td>
<td># females lethal</td>
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<td>146</td>
</tr>
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</table>
Figure 2. Complementation Study-Homozygous Genotypes 25°C

A. \( su(f)^+ / su(f)^+ \)
B. \( yw.f \)
C. \( su(f)^1 / su(f)^1 \)
D. \( su(f)^+ / su(f)^+ \)
E. \( su(f)^1 / su(f)^1 \)
F. \( su(f)R617 / su(f)R617 \)
G. \( su(f)ts67g / su(f)ts67g \)
H. \( su(f)100 / su(f)100 \)
I. \( su(f)ts67g / su(f)ts67g \)
J. \( su(f)^94 / su(f)^94 \)
each, this score ranges from 4.6 to 5.0 and does not show differences with changing temperatures. However, the level of forked suppression does seem to be slightly related to temperature change. The score for the $su(f)^{94}$ mutation goes from 12.0 at 29°C to an average of 10.4 at 21°C while the score for the $su(f)^{100}$ mutation only ranges from scores of approximately 11.4 at 29°C to scores of approximately 10.7 at 21°C. The phenotypes of the mutations are almost identical and so the eye phenotype presented in Figure 2 H is of the $su(f)^{100}$ mutation (raised at 25°C) but is the same for the $su(f)^{94}$ mutation. The opposite is true in Figure 2 J. The bristle phenotype presented is that of the $su(f)^{94}$ mutation (raised at 25°C) and is the same for the $su(f)^{100}$ mutation and the $su(f)^{R617}$ mutation. The eye phenotype of the $su(f)^{R617}$ mutation (raised at 25°C) is shown in Figure 2 F. It is identical to the eye phenotype of $su(f)^{1}$ which the phenotype scores reflect. The bristle scores only range from only 12.0 to 11.4 while the eye phenotype scores range from 3.0 at 29°C to an average of 3.7 at 25°C to an average of 4.6 at 21°C. The scores for the $su(f)^{ts726}$ and $su(f)^{ts678}$ alleles are also consistent with those from previous studies. In both cases, the homozygous females are lethal at 29°C. At 25°C both alleles have moderately $w^a$ enhanced eye phenotype, while showing somewhat more enhancement at 21°C. The numbers reflect that these flies are also much healthier at 21°C than at 25°C. However the forked suppression for both of these homozygous alleles decreases with decreasing temperature. The score ranges from 1.2 to 3.0 for the $su(f)^{ts726}$ allele while the score ranges from 0.1 to 4.2 for the $su(f)^{ts678}$ allele. The phenotype for the $su(f)^{ts678}$ allele (raised at 25°C) is shown in
Figure 3. Complementation Study - Homozygous and Heterozygous Genotypes

A. $su(f)^1 / su(f)^1$ 29°C
B. $su(f)^1 / su(f)^1$ 21°C
C. $su(f)^R617 / su(f)^1$ 25°C
D. $su(f)^R617 / su(f)^1$ 21°C
E. $su(f)^R617 / su(f)^{ts726}$ 29°C
F. $su(f)^94 / su(f)^{ts726}$ 25°C
Figure 2 G and I. This phenotype is very similar to that of the $su(f)_{ts726}$ allele raised at 25°C. Again, these findings are consistent with those previously discussed.

Following documentation of the homozygous phenotypes, the study of the heterozygous females was completed. Individuals were generated containing $su(f)^{94}$, $su(f)^{100}$, or $su(f)^{R617}$ heterozygous with $su(f)^1$, $su(f)_{ts726}$, $su(f)_{ts67g}$, $su(f)^+$, and the $su(f)$ deficiency VE738. These individuals were scored, as described above, and the results are presented in Table 4. When the $su(f)^{94}$ mutation is heterozygous with the $su(f)^1$, $su(f)_{ts726}$, and $su(f)_{ts67g}$ alleles, $su(f)^+$, or the $su(f)$ VE738 deficiency, the eye phenotype scores are roughly the same at all temperatures. In all five heterozygous conditions, the score is approximately 3.0 and does not change with temperature. For reference, a score of 3.0 looks like a $wa$ allele over a white allele. This eye phenotype is seen in Figure 4 E which is $su(f)^{94}/su(f)_{ts67g}$ raised at 21°C. The same finding is true of the $su(f)^{100}$ mutation when heterozygous with the $su(f)^1$, $su(f)_{ts726}$, and $su(f)_{ts67g}$ alleles, or the $su(f)$ VE738 deficiency. (The $su(f)^{100}$ mutation was not scored as a heterozygote with $su(f)^+$.) The reason for the non-changing eye phenotype will be discussed in the Tests on $su(f)$ alleles Section below. However, the bristle score is a much different story. In the $su(f)^{94}/su(f)^1$ female, the bristle score ranges from 6.8 to 10.2. The level of suppression of forked does seem to decrease slightly as the temperature decreases. This change in suppression levels is even greater in $su(f)^{94}/su(f)_{ts726}$, where the score ranges from 6.3 to 10.9 and also in $su(f)^{94}/su(f)_{ts67g}$, where the score ranges from 6.0 to 10.0. Also in $su(f)^{94}/su(f)$
Table 4. Results of the Complementation Study for Heterozygous Genotypes

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Description</th>
<th>29°C</th>
<th>25°C</th>
<th>21°C</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Day 13</td>
<td>Day 16</td>
<td>Day 16</td>
</tr>
<tr>
<td>su(f) 94 / su(f) 1</td>
<td>eye color</td>
<td>2.8</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td># females</td>
<td>117</td>
<td>151</td>
<td>104</td>
</tr>
<tr>
<td>su(f) 94 / su(f) 726</td>
<td>eye color</td>
<td>2.0</td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td># females</td>
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<td>6.3</td>
<td>8.4</td>
</tr>
<tr>
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<td>2.5</td>
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<td></td>
<td># females</td>
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<td>7.1</td>
</tr>
<tr>
<td>su(f) 100 / su(f) 1</td>
<td>eye color</td>
<td>2.0</td>
<td>1.7</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td># females</td>
<td>8.2</td>
<td>5.7</td>
<td>7.6</td>
</tr>
<tr>
<td>su(f) 100 / su(f) 726</td>
<td>eye color</td>
<td>1.5</td>
<td>1.5</td>
<td>2.8</td>
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</tr>
<tr>
<td></td>
<td># females</td>
<td>6.4</td>
<td>6.4</td>
<td>9.4</td>
</tr>
<tr>
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<td>eye color</td>
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<td>2.5</td>
<td>4.0</td>
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<tr>
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<td># females</td>
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<td>6.7</td>
<td>89</td>
</tr>
<tr>
<td>su(f) R617 / su(f) 706</td>
<td>eye color</td>
<td>2.6</td>
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<td>2.9</td>
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<tr>
<td></td>
<td># females</td>
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<td>8.6</td>
<td>773</td>
</tr>
<tr>
<td>su(f) R617 / su(f) 67g</td>
<td>eye color</td>
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<td>3.1</td>
</tr>
<tr>
<td></td>
<td># females</td>
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<tr>
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<td>2.3</td>
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<td>122</td>
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<tr>
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<td>1.5</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td># females</td>
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<td>9.9</td>
<td>9.9</td>
</tr>
<tr>
<td>su(f) + / su(f) 726</td>
<td>eye color</td>
<td>1.5</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td># females</td>
<td>11.2</td>
<td>10.7</td>
<td>11.1</td>
</tr>
<tr>
<td>su(f) 84 / su(f) DIVE738</td>
<td>eye color</td>
<td>X</td>
<td>X</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td># females</td>
<td>X</td>
<td>X</td>
<td>132</td>
</tr>
<tr>
<td>su(f) 100 / su(f) DIVE738</td>
<td>eye color</td>
<td>X</td>
<td>X</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td># females</td>
<td>X</td>
<td>X</td>
<td>7.7</td>
</tr>
<tr>
<td>su(f) R617 / su(f) DIVE738</td>
<td>eye color</td>
<td>X</td>
<td>X</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td># females</td>
<td>X</td>
<td>X</td>
<td>4.8</td>
</tr>
</tbody>
</table>
DfVE738 the bristle phenotype score is an average of 8.0.

Upon seeing this response, it was important to document that $su(f)^{94}$ mutation and the $su(f)^{ts67g}$ and $su(f)^{ts726}$ alleles are recessive. They were analyzed as heterozygotes with $su(f)^{+}$. The eye phenotype score of $su(f)^{94}/su(f)^{+}$ averaged around 3.0 as discussed before, but for $su(f)^{ts67g}/su(f)^{+}$ and $su(f)^{ts726}/su(f)^{+}$ the eye phenotype scores averaged around 1.5 a score representative of no enhancement. For the bristles, the scores for $su(f)^{94}/su(f)^{+}$ averaged around 10.4 while the scores for $su(f)^{ts67g}/su(f)^{+}$ and $su(f)^{ts726}/su(f)^{+}$ averaged around 10.0 and 11.2, respectively. All of these scores are representative of the lack of forked suppression, and therefore the alleles and the mutation are recessive. The recessiveness of both the alleles has been shown previously by Russell (1974) and Lambertsson (1975). Therefore the $su(f)^{94}$ mutation, the $su(f)^{ts67g}$, and $su(f)^{ts726}$ alleles are recessive. However, in $su(f)^{94}/su(f)^{ts67g}$ suppression of the forked phenotype is showing and it decreases with decreasing temperature. Thus the $su(f)^{94}$ mutation and the $su(f)^{ts67g}$ allele do not complement. This indicates they are alleles. This noncomplementing phenotype is shown in Figure 4. First of all, the eye phenotype as described above of $su(f)^{ts67g}/su(f)^{+}$ (raised at 25°C) is shown in Figure 4 A, while the bristle phenotype of $su(f)^{ts67g}/su(f)^{+}$ (raised at 25°C) is shown in Figure 4 B. A less suppressed bristle phenotype of $su(f)^{ts67g}/su(f)^{ts67g}$ (raised at 21°C) is shown in Figure 4 C. This can be compared to Figure 2 I where the bristle phenotype of $su(f)^{ts67g}/su(f)^{ts67g}$ (raised at 25°C) is shown, demonstrating the decreasing suppression with decreasing temperature. The bristle phenotype
Figure 4. Complementation Study - Homozygous and Heterozygous Genotypes

A. su(f)^ts67g / su(f)^+  25°C
B. su(f)^ts67g / su(f)^+  25°C
C. su(f)^ts67g / su(f)^ts67g  21°C
D. su(f)^94 / su(f)^+  29°C
E. su(f)^94 / su(f)^ts67g  21°C
F. su(f)^94 / su(f)^ts67g  29°C
of $su(f)^{94}/su(f)^+$ in Figure 4 D shows the lack of *forked* suppression, thus supporting the $su(f)^{94}$ mutation as recessive. The noncomplementing phenotype of $su(f)^{94}/su(f)^{ts678}$ is shown in Figure 4 E and F. In Figure 4 E the female was raised at 21°C, while the female in 4 F was raised at 29°C showing the strongest suppression of the *forked* phenotype. Individuals of the genotype $su(f)^{94}/su(f)^{ts726}$ (raised at 25°C) is shown in Figure 3 F show a suppressed *forked* phenotype. Identical results are obtained when the $su(f)^{100}$ mutation is analyzed over the $su(f)^{ts678}$ and $su(f)^{ts726}$ alleles. Therefore, these noncomplementing phenotypes of $su(f)^{94}/su(f)^{ts678}$, $su(f)^{94}/su(f)^{ts726}$, $su(f)^{100}/su(f)^{ts678}$, and $su(f)^{100}/su(f)^{ts726}$ demonstrate that both $su(f)^{94}$ and $su(f)^{100}$ are alleles of $su(f)$.

Another mutation that was tested for complementation was the $su(f)^{R617}$ mutation. The eye phenotype of this mutation, is identical to that of $su(f)^{I}$ as stated earlier. As shown in previous complementation studies (J. Girton, Department of Genetics, Iowa State University, personal communication) the level of $w^{a}$ enhancement increases with decreasing temperature. This is true in all of the cases in which the $su(f)^{R617}$ mutation is heterozygous with known $su(f)$ alleles. When the $su(f)^{R617}$ mutation is heterozygous with the $su(f)^{I}$ allele, it shows a great deal of *forked* suppression; the bristle phenotype scores range from 1.2 to 3.2 as shown in Table 4. When the $su(f)^{R617}$ mutation is heterozygous with the $su(f)^{ts726}$ and $su(f)^{ts678}$ alleles, the level of *forked* suppression also decreases with decreasing temperatures as evidenced by the range of bristle phenotype scores, from 0.3 to 7.1 and from 1.3 to 5.7, respectively. When
the su(f)R617 mutation is heterozygous with the su(f) deficiency VE738, the bristle phenotype score is an average of 3.0 showing suppression of the forked phenotype while the eye phenotype score is an average of 3.5. Since the homozygous phenotype of the su(f)R617 mutation shows no forked suppression, and is very different from the homozygous phenotype of the su(f)1 allele, from which the reversion was obtained, it is important to know whether this new homozygous bristle phenotype is due to a change at su(f) or forked. Therefore these heterozygous phenotypes involving the su(f)R617 mutation are very important in determining if this reversion is still an allele of su(f). As shown by the results discussed above, the su(f)R617 / su(f)1, su(f)R617 / su(f)ts726, and su(f)R617 / su(f)ts678 heterozygotes show a noncomplementing suppressor of forked phenotype. These phenotypes are shown in Figure 3. In Figure 3 C and D is su(f)R617 / su(f)1 raised at 21°C and 25°C, respectively. Since the su(f)1 allele is recessive, the su(f)R617 mutation must be deficient at the su(f) locus causing the suppressed phenotype. Thus su(f)R617 is an allele of su(f). Also shown in Figure 3 E is the heterozygote su(f)R617 / su(f)ts726 raised at 29°C. It shows a very suppressed phenotype. In fact the bristles are thinner and smaller than other "suppressed forked" bristles. It has been shown that another phenotype of recessive su(f) alleles is a "Minute " or "missing bristle" effect (Russell, 1974, and Russell et al., 1977). The resulting bristles are smaller and thinner, just like those in Figure 3 E. Sometimes it even looks as if the bristle has "fallen" off resulting in a "stubby" bristle or a socket without a bristle. In Figure 3 E, one of the anterior scutellar bristles is missing although the base remains. Also
missing is one of the anterior dorso-centrals. This effect can also be seen in Figure 3 F in the su(f)^94 / su(f)^ts726 heterozygote, where one of the posterior scutellar bristles is "stub-like" and again in Figure 4 F in the su(f)^94 / su(f)^ts678 heterozygote.

To summarize the complementation studies, it was found that su(f)^94, su(f)^100, and su(f)^R617 are truly alleles of su(f) due to their failure to complement known suppressor of forked alleles. However, the homozygous phenotypes of these alleles are somewhat more complicated than stated above and will be discussed below in the Tests on su(f) alleles Section.

Tests of su(f) with the lozenge gene

Most of the phenotypic analysis of su(f) is based on (1) its ability to enhance w^a due to the termination of transcription as instructed by the copia element (which is responsible for the phenotype of w^a) and (2) the suppression of forked due to the presence of the gypsy element at that locus. How does su(f)^+ affect the transcription of the gypsys and copias at additional loci? An important genetic test of the model of action is to examine the phenotypic effect of su(f) on gypsy and copia insert alleles of a single locus. When the alleles of this single locus are in cis with su(f), are they phenotypically suppressed or enhanced when compared to the phenotype of the allele alone? This type of analysis will result in a better understanding of the trans-acting effect of su(f) on other alleles at other loci.
One such locus to test is *lozenge* (*lz*). According to the literature (Green and Green, 1956) there are two distinct phenotypic classes of *lozenge* alleles, with spontaneous mutants in both classes. The *lz* alleles studied were *lz*^1^, *lz*^34^, and *lz*^37^ which represent Class I and *lz*^36^ which represents Class II. In 1976, Snyder and Smith found that the interaction between *su(f)*^I^ and *lz*^I^ gives rise to a suppressed phenotype with phenol oxidase activity increasing from 17% wild-type activity in *lz*^I^ to 71% wild-type activity in *lz*^I^-*su(f)*^I^. Conversely, *lz*^37^ is phenotypically enhanced and phenol oxidase activity decreases from 94% wild-type activity to 58% wild-type activity in *lz*^37^-*su(f)*^I^.

Also, according to A. Schalet (Synder and Smith, 1976) there is no phenotypic interaction between *lz*^34^ and *su(f)*^I^. The interaction of *lz*^36^ was not reported. If the mutational lesions of the *lz*^I^ or *lz*^37^ alleles are caused by a *gypsy* insertion or by a *copia* insertion, respectively, then the action of *su(f)*^+^ is to positively regulate the transcription of the *gypsy* element consequently increasing transcription of the locus thus suppressing the mutant phenotype, or to negatively regulate the transcription of the *copia* element by causing premature termination of transcription thus enhancing the mutant's phenotype. In Lindsley and Zimm, 1990, it is stated that the *lz*^I^ allele does in fact contain a *gypsy* element. However, the origin of the *lz*^37^ allele is listed simply as spontaneous. It is not known whether a *copia* element resides in the gene giving rise to the *lz*^37^ allele (M. Green, University of California, Davis, California, personal communication). By studying the enhancement and the suppression of a single mutant phenotype instead of enhancement at one
locus and suppression at another, the activity of $su(f)^+$ at the transcriptional level should be better understood.

The results from this study are presented in Figure 5. All of the genotypes presented were raised at 25°C. The $lz^{37}$ allele in cis with the $f^I$ allele is shown in Figure 5 A. This is the control phenotype for the $lz^{37}$ allele. $Lz$ has many phenotypic effects and this study focused on the eye phenotype. The $lz$ eye is somewhat ovoid in shape, the surface has a slightly roughened and glistening appearance, referred to as glossy, and the red eye pigment is uniformly distributed. The male with the $lz^{37} f^I \ su(f)^+$ / $Y$ genotype is shown in Figure 5 B. The eye phenotype appears "more mutant", thus an enhanced phenotype. The shape of the eye is more ovoid than the control, and the surface shows a more glossy appearance. Neither the $su(f)^{94}$ nor the $su(f)ts^{678}$ alleles seemed to affect the phenotype of $lz^{37}$. In the case of the $lz^I$ allele, the control phenotype is presented in Figure 5 C. The genotype shown is a $lz^I f^I / Y$ male. The eyes are ovoid in shape, the surface is glossy, and the pigment is evenly distributed. A $lz^I f^I \ su(f)^+$ / $Y$ male is shown in Figure 5 D. The eye phenotype appears "less mutant"; its eyes are less ovoid, and the surface is less glossy showing a suppressed phenotype. Shown in Figure 5 E, is a $lz^I f^I \ su(f)ts^{678} / Y$ male. The phenotype is slightly suppressed. The eyes are less ovoid, however, the surface does appear as glossy as the control phenotype. The exact same phenotype was seen for the $lz^I f^I \ su(f)^{94} / Y$ male. This is an expected result, since both the $su(f)ts^{678}$ and $su(f)^{94}$ alleles are weak suppressors of $forked$, it would be assumed that they would be weak suppressors of $lz^I$. In Figure 5 G is the $lz^{36} f^I / Y$ genotype. The eye
Figure 5. *Lozenge Study - all Genotypes - 25°C*

A. $lz^{37} f^{1} / Y$
B. $lz^{37} f^{1} su(f)^{1} / Y$
C. $lz^{1} f^{1} / Y$
D. $lz^{1} f^{1} su(f)^{1} / Y$
E. $lz^{1} f^{1} su(f)^{1}s67g / Y$
F. $lz^{34} f^{1} su(f)^{94} / Y$
G. $lz^{36} f^{1} / Y$
H. $lz^{34} f^{1} / Y$
phenotype shows a definite ovoid shape, the surface is very glossy, and the pigment is not uniformly distributed. It is concentrated at the periphery of the eye and is referred to as "spectacle" (Lindsley and Zimm, 1990). None of the \textit{su(f)} alleles tested (\textit{su(f)}\textsuperscript{I}, \textit{su(f)ts67g}, and \textit{su(f)}\textsuperscript{94}) interacted with the \textit{lz36} allele. This was the "unknown" allele, that is, interaction with \textit{su(f)} alleles has never been reported for this \textit{lz} allele. Finally in Figure 5 H is the \textit{lz34 f}\textsuperscript{I} / \textit{Y} male. Its eye phenotype shows a strong ovoid shape, a glossy surface, and the spectacle characteristic. In Figure 5 F is the \textit{lz34 f}\textsuperscript{I} \textit{su(f)}\textsuperscript{94} / \textit{Y} male. Its phenotype shows the same strong ovoid shape and a very glossy surface. However, the spectacle phenotype is much stronger. After observing several generations of these males, this enhanced phenotype remains consistent. The allele is not a strong enhancer of \textit{w}\textsuperscript{d} (discussed in detail below) which makes this result puzzling. Neither the \textit{su(f)}\textsuperscript{I} allele nor the \textit{su(f)ts67g} allele showed any interaction with the \textit{lz34} allele.

This locus is currently being further tested. The \textit{su(f)}\textsuperscript{R918} and \textit{su(f)ts726} alleles are each being recombined with the \textit{lzI} and \textit{lz37} alleles. These crosses are being completed in order to try to obtain a more complete picture of the enhancement and suppression effects that \textit{su(f)} alleles have on the \textit{lz} alleles. Thus far, the hypothesis seems to still be in place, that is, the suppression and enhancement effects of \textit{su(f)} alleles on a single locus can be tested, and show different results depending on (1) the locus selected, and (2) the origin of the associated alleles.
Tests on *su(f)* alleles

Confirmation of allelism can be accomplished several ways. A standard technique is the study of complementation as discussed above. However, the enhancement and suppression phenotypes of many *su(f)* alleles are almost totally dependent upon the reporter alleles, *w^a* and *f^l*. The resulting phenotypes of these mutations in the presence of *su(f)* alleles is deemed "the *su(f)* phenotype". In order to fully determine the exact phenotype of the three *su(f)* alleles, *su(f)^94*, *su(f)^100*, and *su(f)^R617*, it was necessary to "break apart" the phenotype, and then "rebuild" the phenotype.

The suppression of the *forked* phenotype for the *su(f)^94* and *su(f)^100* alleles has been fully demonstrated in the Complementation Studies Section above. However, the enhancement of *w^a* phenotype was called into question. The eye phenotype does not change with changing temperature. When these mutations were first isolated they looked exactly like the *su(f)^94/* *su(f)*^DfVE738* and *su(f)^100/* *su(f)*^DfVE738* heterozygous females as described in Table 4 and in the Complementation Studies Section above. The *w^a* enhancement was moderate, as was the suppression of *forked* phenotype. Also, as stated above, it was not until after many generations of maintaining the stocks *y^2 w^a f^l su(f)^94* / *FM7* or *y^2 w^a f^l su(f)^100* / *FM7* females *X FM7* / *Y* males that the homozygous *y^2 w^a f^l su(f)^94* / *y^2 w^a f^l su(f)^94* and *y^2 w^a f^l su(f)^100* / *y^2 w^a f^l su(f)^100* females appeared. Both strains of homozygous females appeared to show extreme *w^a* enhancement, that is, the eyes were almost completely white, and less *f* suppression. Several hypotheses could explain the mutant phenotype. The first is that a
mutation at $su(f)$ changed the ability of $su(f)^+$ to enhance $w^a$ such that $w^a$ is always enhanced. The second is that the mutational event really occurred at $w^a$ and not at $su(f)$. Several crosses were done to test the question of whether this phenotype is due to a new mutation at white or at suppressor of forked. It was important to know if this phenotype was a result of a true mutation at $su(f)$ because none of the alleles in the collection of $su(f)$ alleles have a phenotype similar to the homozygous $su(f)^{94}$ and $su(f)^{100}$ phenotype.

Since homozygous $su(f)^{94}$ and $su(f)^{100}$ are strong enhancers of $w^a$ but weak suppressors of forked, their phenotype is different from other $su(f)$ alleles. Other $su(f)$ alleles are either strong enhancers and strong suppressors, e.g., $su(f)^1$, weak enhancers and weak suppressors, e.g., $su(f)^{ts67g}$, or weak enhancers, and strong suppressors, e.g., $su(f)^{pb}$. Thus far in our studies, the evidence is strong, but not convincing, that the enhancing of $w^a$ and the suppression of forked functions are indeed separable. Therefore the analysis of these two alleles will help us to further test and refine our current model of action.

In order to test this strong effect on the $w^a$ allele several crosses were made. After the heterozygous virgin $y^2 w^a^*f^1 su(f)^{94}/+ \text{ females were mated to } +/Y \text{ males, recombinant males were selected. The phenotype of question was the } y^2 w^a^*/Y \text{ males. They did not show the forked phenotype and therefore the } su(f) \text{ allele should not be present (except for rare double crossovers).}$ Now that the $w^a^*$ allele was separated from the $su(f)$ allele, did the non-enhanced $w^a$ phenotype return? The answer is no. Out of all the white eyed males, approximately 25% should be due to the
parental chromosome, $y^2 w^a f^l su(f)^{94} / Y$, while another approximate 25% would be the $y^2 w^a / Y$ male, since the crossover frequency between $w^a$ and $f^l$ is 50%. There were 190 males with white eyes and forked bristles counted while there were 182 males with white eyes and non-forked bristles. The nonenhanced $w^a$ phenotype was never regained after "breaking apart" the chromosome. However, this mutation has been shown to be an allele of $su(f)$ with complementation tests due to the suppression of forked phenotype. It also shows a RFLP using probes that are known to be clones of $su(f)$ due to in situ hybridization to polytene chromosome results, discussed below. Therefore, the strong enhanced $w^a$ phenotype of this mutation was not due to a mutation at $su(f)$ but a mutation at white. In the original mutagenesis experiment, a double event must have occurred giving rise to the strong white eye phenotype, and a weak mutation at $su(f)$.

Following this finding, recombinant males with wild type eyes and forked bristles, presumably the genotype $f^l su(f)^{94} / Y$, were singly mated to homozygous $y^2 w^a f^l \cdot y^+ / y^2 w^a f^l \cdot y^+$ virgin females. There is a 10% map distance between $f^l$ and $su(f)$, therefore, among this phenotype of males, approximately 5% might not carry the $su(f)^{94}$ allele. Due to this percentage, 24 of these males were singly mated. Heterozygous $y^2 w^a f^l \cdot y^+ / f^l su(f)^{94}$ virgin females were collected in the next generation from each of the 24 lines and mated to $+/ Y$ males. Recombinant males that showed the $w^a$ and $f^l$ markers and were not $y^+$ were selected. Now it was important to ask if these males showed any $w^a$ enhancement since a new $w^a$ allele was brought together with the $su(f)$ allele. In each of the 24 lines the phenotype of the males was very slight $w^a$ enhancement (when compared
to the $y^2 w^a f^I / Y$ genotype) and slight to moderate $f$ suppression. These recombined chromosomes were scored in the same manner as the complementation studies scoring. Overall the eye phenotype was approximately a score of 1.8 while the bristle suppression phenotype was approximately a score of 9.5. Therefore, in the collection of $su(f)$ alleles, these alleles would be classified as very weak $su(f)$ alleles based on their ability to enhance $w^a$ and suppress $f^I$.

However, one question remained in the pursuit of fully characterizing the phenotype of these alleles. Was the RFLP still present after "breaking apart" and "rebuilding" the chromosome? Three recombinant males ($y^2 w^a f^I su(f)_{94} / Y$) like those just described were isolated from each of the 24 lines and were singly mated to $y^2 w^a f^I su(f)_{ts726} / FM7$ virgin females at 29°C. The expected female progeny are $y^2 w^a f^I su(f)_{94} / y^2 w^a f^I su(f)_{ts726}$ and $y^2 w^a f^I su(f)_{94} / FM7$, while the male progeny are $y^2 w^a f^I su(f)_{ts726} / Y$ and $FM7 / Y$. The $ts726$ male is lethal at 29°C and the $FM7$ male is discarded. The $y^2 w^a f^I su(f)_{94} / y^2 w^a f^I su(f)_{ts726}$ female is observed for the same noncomplementation pattern described in the Complementation Studies Section above. If noncomplementation for the suppression of forked phenotype occurs, then the $su(f)_{94}$ allele is present. The sibling $y^2 w^a f^I su(f)_{94} / FM7$ females are used for DNA isolation and a Southern hybridization experiment performed to see if the RFLP remained. These results are currently being completed. One of the objectives of this project, in its initial stages, was to detect molecular lesions from $su(f)$ alleles and to correlate the lesion to the mutant phenotype. The $su(f)_{94}$ and $su(f)_{100}$ alleles became the only hope of
accomplishing this goal in the remaining time. Therefore, this thorough phenotypic and molecular analysis demonstrates the ability to completely characterize a new allele of the \textit{su(f)} gene.

The \textit{su(f)}\textsuperscript{R617} allele was tested in a similar manner. As stated earlier, it was isolated as a reversion of \textit{su(f)}\textsuperscript{I}. Its homozygous phenotype showed extreme \textit{w}\textsuperscript{a} enhancement, just like \textit{su(f)}\textsuperscript{I}, but very little \textit{f} suppression, unlike \textit{su(f)}\textsuperscript{I}. The bristles are not suppressed, even at the 29\textdegree C temperature where the suppression phenotype is normally strongest. The eye phenotype shows strong enhancement of \textit{w}\textsuperscript{a}. The enhancement is weakest at 29\textdegree C and strongest at 21\textdegree C, showing almost complete white eyes. A recombination experiment helped to differentiate between two alternative hypotheses about the origin of this mutation. The first hypothesis is that the \textit{gypsy} element located in the \textit{forked} gene resulting in the \textit{f}\textsuperscript{I} allele (Parkhurst and Corces, 1985) excised imperfectly. This excision event would render the forked locus unmodifiable by the \textit{su(f)}\textsuperscript{I} product, therefore no suppression could take place. An imperfect excision also would not return the \textit{forked} locus to wild type, i.e., straight bristles, but leave it mutant, resulting in nonsuppressible forked bristles.

The second hypothesis was that the reversion event caused a change in the portion of the \textit{su(f)} product which is responsible for suppression. If such a change occurred, the \textit{su(f)} product is no longer able to interfere with transcription at the \textit{gypsy} LTR, resulting in loss of suppression of the forked phenotype, i.e., forked bristles. Recombinant males were selected from the cross of \textit{y}\textsuperscript{2} \textit{wa f}\textsuperscript{I* su(f)}\textsuperscript{R617} / \textit{y}\textsuperscript{2} \textit{wa f}\textsuperscript{I} \textit{y+} females and + / \textit{Y} males. All non-\textit{y+} males were examined. The large majority of these
males carried the $y^2 w^a f^1* su(f)^{R617}$ parental chromosome and showed the *forked* phenotype. However, 10% of these males should be a product of a crossover between $f^1$ on the $y^2 w^a f^1* y^+$ chromosome and $su(f)^{R617}$. These males contain the $su(f)^{R617}$ allele and a new $f^1$ allele in which the presence of the *gypsy* element is certain. Do these males exhibit the suppressed *forked* phenotype? The answer is no. Out of 316 males screened with the non-$y^+$ phenotype, 56 showed a suppressed *forked* phenotype. Therefore, the $su(f)^{R617}$ allele does contain the ability to suppress *forked* and therefore the reversion phenotype of nonsuppressed bristles was due to the excision of the *gypsy* element from $f^1$ rendering it unmodifiable by $su(f)$. However, as discussed in the Complementation Studies Section, the $su(f)^{R617}$ allele exhibits noncomplementing phenotypes when heterozygous with the $su(f)^1$, $su(f)^{ts726}$, and $su(f)^{ts678}$ alleles. The pattern of suppression of *forked* bristles in these heterozygotes is different from the pattern seen in the $su(f)^1 / su(f)^1$, the $su(f)^1 / su(f)^{ts726}$, or the $su(f)^1 / su(f)^{ts678}$ genotypes. Therefore, the reversion event generating the $su(f)^{R617}$ allele was not only an excision of the *gypsy* element at $f^1$ but a change at $su(f)$ resulting in a new pattern of *forked* suppression in the heteroallelic genotypes studied.

**In situ hybridization to polytene chromosomes**

The purpose of this *in situ* hybridization to salivary chromosomes is to determine if the cloned $su(f)$ DNA actually hybridizes to the $su(f)$ locus.

The genotypes used for this experiment were *Ore R, Dp (1:2) B^s / Cy*, and *Dp (1:3) B^s*. The *Ore R* strain was selected as the wild type control. In the
**Dp (1:2) B\(^s\) / Cy** strain, the second chromosome carries a fragment of \(B^S y^+\) with the \(B^S\) marker attached to the 2L tip. The **Dp (1:3) B\(^s\)** strain carries a duplication with \(B^S\) and some heterochromatin on the 3L tip. The \(B^S\) fragment carries a duplication of \(su(f)^+\). Therefore a duplication of \(su(f)^+\) has been translocated to both the 2L tip in one strain and to the 3L tip in the other. These translocated duplications made the detection of hybridized probe DNA much easier. Figure 6 shows an enlarged drawing of the proximal portion of the X chromosome. As indicated, \(su(f)\) is located at approximately 20 C or map distance 65.9. This location is at the base of the X chromosome.

The two probes that were hybridized to each of the three strains were a 6.1 kb Hind III fragment isolated from the pX-3-3 subclone, eliminating the P element portion. The other probe was the linearized pR-9-42 subclone containing the Eco RI 3.2 kb fragment. Both fragments were isolated and labelled as described in the Materials and Methods Section. The purpose of hybridizing the Hind III 6.1 kb fragment was because it contains genomic \(su(f)\) DNA from the region known to be transcribed (K. O'Hare, Imperial College, London, England, personal communication). This probe should hybridize only to unique \(su(f)\) DNA. The second probe contains genomic \(su(f)\) DNA that is part of the homologous repeated DNA as indicated by the dashed line in Figure 10. These two homologous regions in the \(su(f)\) region indicated in Figure 10 are highly conserved at least with respect to one another and among \(su(f)\) alleles. The purpose of this probe was to determine if these regions are conserved any where else in the *Drosophila* genome. This was an approach suggested by K. O'Hare.
Figure 6. Genetic and Salivary Chromosome Maps of the Proximal Portion of the X Chromosome of Drosophila melanogaster
(personal communication) to determine the nature of these regions. Perhaps the hybridization of this probe will help elucidate why this region seems so highly conserved at the DNA level and possibly further determine the nature of the euchromatic/ heterochromatic border, so near su(f).

The results of hybridization when the Hind III 6.1 kb probe was used are presented in Figures 7 and 8. In Figure 7 C the strain from which the chromosomes were isolated is the \textit{Dp (1:2) Bs}. The region of hybridization that is shown is the centromere region. This region is expected to hybridize as well as the region of the 2L tip. This hybridization is shown in Figure 7 D. The 2L tip shows a large addition to the telomere which hybridizes to this probe. When the chromosomes are isolated from the \textit{Dp (1:3) Bs} strain, the probe hybridizes to the centromere region, to the 3L tip, and not to the 2L tip as shown in Figure 8 C. The hybridization to the two different telomeres in the two different strains provide controls for each other. This pattern is seen again at a higher magnification in Figure 8 E. The arrow on the left is pointing at the hybridization at the 3L tip, while the arrow on the right is pointing at the 2L tip which is not hybridizing. The results for hybridization of this probe to chromosomes isolated from the \textit{Ore R} strain are not shown, but shows hybridization only to the centromere and not to either the 2L or the 3L tip.

When the probe containing the Eco RI 3.2 kb fragment hybridizes to the chromosomes of any of the strains, the most striking result is that there is not hybridization to many locations throughout the genome. In fact, the probe hybridizes with the same pattern as the previous probe. In Figure 7
Figure 7. *in situ* Hybridization of *su(f)* probes to salivary gland chromosomes

A. *Dp 1:2 Bs / Cy*  Probe Eco R I 3.2 kb
B. *Dp 1:2 Bs / Cy*  Probe Eco R I 3.2 kb
C. *Dp 1:2 Bs / Cy*  Probe Hind III 6.1 kb
D. *Dp 1:2 Bs / Cy*  Probe Hind III 6.1 kb
E. *Dp 1:3 Bs*  Probe Eco R I 3.2 kb
A, the probe is hybridizing to the centromere as it is in Figure 8 D. However, in Figure 7 A it is clearly shown that the probe hybridizes to a particular arm, not just the region in general. The arm it is hybridizing to is the X chromosome, the rest of which is not shown due to the high magnification. The strain of chromosomes in Figure 7 A is $Dp\ (1:2)\ B^s$ and is $Dp\ (1:3)\ B^s$ in 8 d. The probe is seen hybridizing to the 2L tip of a chromosome from the $Dp\ (1:2)\ B^s$ strain in Figure 7 B. In Figure 7 E the hybridization to chromosomes from the $Dp\ (1:3)\ B^s$ strain is to the centromere as indicated by the arrow in the upper left corner. The 2L tip, which is not hybridizing, as expected, is also shown by the arrow in the lower right corner in Figure 7 E. Chromosomes from the $Dp\ (1:3)\ B^s$ strain in both Figure 8 A and B show hybridization to the 3L tip. In addition to the hybridization to the 3L tip in Figure 8 A, the 2L tip (indicated by the upper arrow) not hybridizing.

The hybridization pattern is consistent with these cloned fragments being genomic clones of $su(f)$. The highly repeated region found in the Eco RI 3.2 kb fragment shows the same hybridization pattern as the Hind III 6.1 kb probe. One possibility is that this highly repeated sequence is associated with the euchromatic / heterochromatic border region. In summary, these probes show a hybridization pattern which is consistent with the hybridization pattern expected for suppressor of forked in these strains.
Figure 8. *in situ* Hybridization of *su(f)* probes to salivary gland chromosomes

A. *Dp 1:3 B*<sup>s</sup>  Probe Eco R I 3.2 kb
B. *Dp 1:3 B*<sup>s</sup>  Probe Eco R I 3.2 kb
C. *Dp 1:3 B*<sup>s</sup>  Probe Hind III 6.1 kb
D. *Dp 1:3 B*<sup>s</sup>  Probe Eco R I 3.2 kb
E. *Dp 1:3 B*<sup>s</sup>  Probe Hind III 6.1 kb
Molecular Genetic Experiments

Subcloning of \( \lambda 252.512 \)

The 16.5 kb \( su(f) \) insert DNA was originally cloned into EMBL4 by K. O'Hare (personal communication). The clone, designated as \( \lambda 252.512 \), and a restriction map of the clone was kindly provided (Figure 9). Subcloning the DNA into smaller subunits was a reasonable goal in order to begin this project, since the unique \( su(f) \) DNA was 16.5 kb in length and maintained in a lambda vector. Maintaining the \( su(f) \) fragments in plasmid vectors made the DNA isolation much easier. Also, the smaller size of the insert DNA resulted in the subclones being much more manageable for use as probes. Accordingly, subcloning of the locus was undertaken. The first subclone contains a 4.4 kb insert from the Xho I site at -6.4 to the Xho I site at -2.0, and is designated as pX-9-2. The second subclone contains a 2.7 kb insert from the Xho I site at -2.0 to the Xho I site at 0.75 in the P-element, and is designated as pX-3-3. The third subclone contains a 3.2 kb fragment from the Eco RI site at +0.2 to the Eco RI site at +3.4, and is designated as pR-1-2. The fourth subclone contains a 5.3 kb fragment from the Eco RI site at +3.4 to the Eco RI site used to clone the fragment into EMBL4 (at +8.7), and is designated as pR-9-42. These four subclones cover the entire 16.5 kb clone with the exception of 750 base pairs (bp) in the P-element and were therefore suitable for use as probes in the \( su(f) \) allele RFLP study discussed later.

Southern blot analyses was very difficult when the pX-3-3 subclone was used as a probe. The non-specific hybridization and high background did
Figure 9. Restriction Map of λ252.512 Genomic Clone and Corresponding Probes
Restriction Map of Drosophila
DNA Insert in X.252.512

co-ordinate system

P-element: - 1 KB

B: BamHI  H: Hind III  X: Xho I
R: EcoRI  S: SalI  A: XbaI

PROBES

Xho I fragment 4.4 KB

X-H fragment 1.9 KB

P Element LTR 0.84 KB

EcoRI fragment 3.2 KB

EcoRI fragment 5.3 KB
not allow for the detection of individual bands. Since the su(f) fragment of
the pX-3-3 subclone contained a portion of a P-element, it was concluded
that it must be hybridizing nonspecifically to sequences of P-elements
throughout the genome. Therefore, by using the GENECLEAN™ kit as
described in the Materials and Methods, the portion of the subclone that
was unique su(f) DNA was isolated. The pX-3-3 subclone was double
digested with Xho I and Hind III thus separating the vector band (4.2 kb),
the 0.8 kb band that was partial P-element, and the 1.9 kb band that was
unique su(f) DNA. The X-H 1.9 kb band was then used as a probe in the
genomic Southerns. Similarly, the other three subclones, pX-9-2, pR-9-42,
and pR-1-2 were used as probes on the genomic Southerns. In a later part
of the study, insert-only DNA was isolated from these subclones also using
the GENECLEAN™ kit, as described in the Materials and Methods, and
then used as probes.

Restriction mapping of genomic su(f) DNA

A modified restriction map of the region was established by restricting
y^2 w^y f y^+ FM7 genomic DNA with seven different restriction enzymes
including Bam HI (B), Eco RI (R), Hind III (H), Pst I (P), Sal I (S), Xba I
(A), and Xho I (X). DNA from two other genotypes was included in the
study serving as a negative and positive control. As a negative control,
su(f)^hd252 FM7 DNA, was analyzed because of its known restriction
patterns. Since both the su(f) wild type and the su(f)^hd252 mutant
genotypes were balanced over FM7, genomic DNA from FM7/Y was used
as a positive control. The map allows for the comparison of restriction
patterns between wild type \( su(f) \) DNA and \( su(f) \) alleles. The following restriction digests were analyzed on each of the three DNA types (according to the above abbreviations): single enzyme digests were B, R, H, P, S, A, X; double enzyme digests were B and R, B and H, B and P, B and S, B and A, B and X, R and H, R and P, R and X, H and P, H and A, H and X, P and S, P and A, P and X, S and A, and S and X.

The insert-only DNA sequences from the four subclones, pX-9-2, pX-3-3, pR-9-42, and pR-1-2, were used as probes. As a result, new restriction sites have been mapped in this region. The modified restriction map is an expansion on the restriction map of the clone provided by O'Hare et al. and is shown in Figure 10. Previously, the cloned \( su(f) \) region was confined to the limits of the 16.5 kb insert of \( \lambda252.512 \). With this mapping, the region of unique suppressor of forked DNA that has been mapped has been extended by 11.1 kb for a total of 27.6 kb. Fifteen new restriction sites have been mapped and are indicated by circles around the restriction enzyme letter in Figure 10. The map of \( su(f) \) from genomic DNA matches the map of the cloned \( su(f) \) DNA fairly closely, with a few minor changes. For example, in the original map of the \( su(f) \) clone, the Bam HI and Hind III site to the "left" of the P-element are shown at the same site in Figure 9. In actuality, the sites are about 0.05 kb apart as shown in Figure 10.

A region of homology exists between the two areas of the clone underlined in dashed lines in Figure 10. This homology was deduced because of the cross hybridization of the Xho I 4.4 kb insert-only probe and the Eco 3.2 kb insert-only probe. This finding was not surprising since the region of \( su(f) \) is just proximal to the heterochromatic border, a
Figure 10. Modified Restriction Map of su(f). Restriction Enzyme Abbreviations
A=Xba I, B=Bam HI, H=Hind III, P=Pst I, S=Sal I, R=Eco RI, X=Xho I
Modified $su(f)$ Restriction Map

In the diagram:

- **Probes**
  - $3.2$ unit
  - $1.9$ unit
  - $4.4$ unit

- **Enzyme Abbreviations**
  - $R$ = Eco RI
  - $X$ = Xho I

- **Markers**
  - $-2$ to $+12$
  - $+14$

- **Other**
region known to contain repeated DNA sequences. These 2 cross hybridizing regions were also found by K. O'Hare (personal communication) and seem to be very conserved. The regions of homology are detected by the Xho I 4.4kb probe and the Eco RI 3.2 kb probe. These regions of homology are from the X(+6.4) restriction site to the H(+4.35) site and from the R(-0.2) site to the R(-3.4) site. The actual size and number of repeated sequence could be somewhat different, so to determine their exact nature, further restriction mapping will be required. In every case where bands would be expected to appear due to cross-hybridization of the probes, the predicted results were found. For example, when DNA from several alleles is digested with Xho I and probed with Eco RI 3.2 kb insert-only DNA, a large band (from the Xho I +2.0 toward the "left of the map" past -13.6) should hybridize. This band does appear, but each allele shows a very strong hybridization to the Xho 4.4 kb band which should not "normally" hybridize to this probe. This shows that the Eco RI kb probe is very similar to the Xho I 4.4 kb probe. This cross-hybridization is very strong which indicates a highly conserved homology between these two regions. Further tests to were done to determine the frequency of these repeats throughout the genome using in situ hybridization to salivary chromosomes and was discussed above.

A recent report published in the Drosophila Information Service (Lindsley and Zimm, 1990) states that the repeats are from -32.0 to -29.4, from -23.4 to -19.9, from -10.2 to -7.8, from -7.4 to -6.6, from -5.0 to -2.0, and from +4.9 to +19.0. It also reports that the positive values are to the right, toward the centromere as depicted in Figure 10. This is why the
map of the clone in Figure 9 is opposite in orientation, due to the fact that the orientation of the clone with respect to the centromere was not known in the early stages of this research.

**Restriction Fragment Length Polymorphism study**

A restriction fragment length polymorphism (RFLP) is a DNA fragment size difference between two alleles of the same gene. It is detected when these alleles are restricted with the same enzyme and hybridized with the same probe. Many alleles of *su(f)* were examined to determine RFLPs using the technique of Southern Hybridization. They were probed with all four unique *su(f)* fragments and an LTR of a cloned P-element. This probe was isolated from the π225.1 plasmid clone, in which a full length P-element is cloned into pBR322 (O'Hare and Rubin, 1983). The clone was digested with Hind III separating a 0.84 kb fragment that is part of the P-element LTR from the vector DNA. This fragment was purified using the GENECLEAN™ kit. The DNA of each allele was cut with seven different restriction enzymes. They were Bam HI (B), Eco RI (R), Hind III (H), Pst I (P), Sal I (S), Xba I (A), and Xho I (X). All of the DNA was isolated from heterozygous females for the *su(f)* allele and the FM7 balancer chromosome. FM7 DNA was always included as a control and was isolated from *FM7/Y*. Another control was DNA from the strain *y^2 w^d f^d \cdot y^+*, which is the strain into which all *su(f)* alleles were crossed. Therefore, all alleles were in the same genetic background. The *su(f)* alleles and controls from which DNA was isolated, digested and analyzed were: Ore R (wild type), *y^2 w^d f^d \cdot y^+ (su(f) wild type), FM7/Y*,
su(f)EMS 5, su(f)EMS 11, su(f)EMS 15, su(f)mad ts, su(f)C11, su(f)K5, su(f)R918, su(f)\textsuperscript{3a}, su(f)hd252, su(f)hd71, su(f)ts726, su(f)ts678, su(f)\textsuperscript{1}, su(f)\textsuperscript{886}, su(f)IB116, su(f)\textsuperscript{100}, su(f)\textsuperscript{94}, su(f)D13, and su(f)\textsuperscript{126}.

The DNA was analyzed on 20 x 20 cm nitrocellulose membranes in three sets. The sets are indicated as B, C, and D and the DNA was grouped into sets as follows:

<table>
<thead>
<tr>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>y\textsuperscript{2} w\textsuperscript{a} f\textsuperscript{1} + * y\textsuperscript{+}</td>
<td>y\textsuperscript{2} w\textsuperscript{a} f\textsuperscript{1} + * y\textsuperscript{+}</td>
<td>y\textsuperscript{2} w\textsuperscript{a} f\textsuperscript{1} + * y\textsuperscript{+}</td>
</tr>
<tr>
<td>FM7/ Y</td>
<td>FM7/ Y</td>
<td>FM7/ Y</td>
</tr>
<tr>
<td>hd71</td>
<td>R918</td>
<td>Ore R</td>
</tr>
<tr>
<td>EMS 5 (1st)</td>
<td>IB116</td>
<td>hd252</td>
</tr>
<tr>
<td>EMS 11</td>
<td>3a</td>
<td>726</td>
</tr>
<tr>
<td>EMS 15</td>
<td>126</td>
<td>67g</td>
</tr>
<tr>
<td>mad ts</td>
<td>C11</td>
<td>su(f)\textsuperscript{1}</td>
</tr>
<tr>
<td>100</td>
<td>K5</td>
<td>EMS 5 (2nd)</td>
</tr>
<tr>
<td>94</td>
<td>D13</td>
<td>886</td>
</tr>
</tbody>
</table>

After establishing the modified restriction map, it was important to return to the original autoradiograms and determine if any RFLPs could be found among the su(f) alleles. The su(f) alleles from this group which showed RFLPs were su(f)hd252, su(f)\textsuperscript{94}, and su(f)\textsuperscript{100}. The RFLPs associated with the su(f)hd252 allele were expected. This allele contains a P-element and was the source of DNA from which su(f) was cloned by P-element transposon tagging (O'Hare, Imperial College, London, England, personal communication). The original restriction map of the clone, \textlambda 252.512, included the restriction sites located within the P-element. The work that is presented in Figures 11 and 12 confirms that these restriction
sites are present and that there were no additional sites located within this 1.2 kb P-element insertion.

In both Figure 11 and Figure 12, DNA from the genotype su(f)hd252/FM7 was isolated and restricted with the enzymes indicated. The DNA is organized in groups of three; that is, in lane 1 is y2 wa f1/y+/FM7, in lane 2 is FM7/Y, and in lane 3 is su(f)hd252/FM7. This sequence of genotypes is then repeated in lanes 4-6, 7-9, 10-12, 13-15, and 16-17. The probe used in Figure 11 is the X-H 1.9 kb fragment. Restricting the DNA with Eco RI and Pst I as in lanes 1 and 2, a 2.8 kb band hybridizes, which is the expected wild type pattern. In lane 3, both a 2.8 kb band and a 4.0 kb band hybridize. The 2.8 kb band is due to the presence of the FM7 chromosome and the 4.0 kb band is a result of the 1.2 kb insertion in the su(f)hd252 chromosome. In the original restriction map of the clone, the P element contained neither Eco RI nor Pst I sites. The result in Figure 11 confirms that finding. When the DNA is restricted with Bam HI and Eco RI as in lanes 4-6, a 4.45 kb band hybridizes in the lanes with wild type DNA and an additional 5.65 kb band in the lane containing su(f)hd252 DNA, also indicating a 1.2 kb insertion. When the DNA is restricted with Hind III and Eco RI as in lanes 7-9, a 2.4 kb band is expected to hybridize in the lanes containing the wild type DNA. The fragment the probe hybridizes to is from the Hind III site at +2.2 to the Eco RI site at -0.2 (Figure 10). In lane 9, an additional 2.1 kb band hybridizes showing that there is an additional Eco RI or Hind III site within the element. According to the original map, the P-element is inserted 0.1 kb to the right of the Sal I site at map position 0 and a Hind III site is located at this same
Figure 11. Restriction Fragment Length Polymorphism Analysis on the $su(f)^{hd252}$ allele using the Xho I - Hind III 1.9 kb Probe

Lanes 1, 4, 7, 10, 13, and 16 contain $y^2 \, w^a \, f^1 \cdot y^+ / FM7$ DNA

Lanes 2, 5, 8, 11, 14, and 17 contain $FM7 / Y$ DNA

Lanes 3, 6, 9, 12, 15, and 18 contain $y^2 \, w^a \, f^1 \, su(f)^{hd252} / FM7$ DNA
position. In the *su(f)*^hd252* DNA, a smaller Hind III (+2.2) to the P-element Hind III site fragment would hybridize to the probe resulting in a 2.1 kb band. This Hind III site is designated as P-element Hind III (0.0). The presence of this same Hind III site is demonstrated in lanes 10-12. The DNA is restricted with Hind III and Xho I. A fragment from Xho I(+2.0) to Hind III (-3.2) should hybridize to the probe yielding a 5.2 kb band which is shown in the lanes containing wild type DNA. The *su(f)*^hd252* DNA in lane 12 shows a 1.9 kb hybridizing band which results from the Xho I(+2.0) to the P-element Hind III (0.0) fragment confirming the location of this P-element Hind III site. In lanes 13-15, the DNA is digested with Xho I and Pst I. The expected hybridization pattern for the wild type DNA is a 9.5 kb band. The hybridizing fragment is from the Xho I site at +2.0 to the Pst I site at -7.5. However in the lane with *su(f)*^hd252* DNA (lane 15) a 2.65 kb band is hybridizing. This indicates the presence of either an Xho I or a Pst I site within the element. When the DNA was cut with Eco RI and Pst I in lane 3, neither enzyme cut within the element. Therefore, an Xho I site must be located at P-element Xho I (0.75). In lanes 16-18, the DNA is cut with Xho I and Bam HI. The expected hybridizing band for the wild type DNA is 6.8 kb. If the presence of the P-element Xho I site is correct, then a 2.65 kb band should also be hybridizing in lane 18. The band in lane 18 looks to be a smaller size than the one in lane 15, which is also 2.65 kb. Due to the slant of the gel when it was blotted a general curve is noted in this figure. When this experiment was repeated, the size of the RFLP for *su(f)*^hd252* DNA when digested with these same two enzymes was indeed 2.65 kb.
The study of the RFLPs associated with the \textit{su(f)^{hd252}} allele was continued in Figure 12. The probe used was the Eco RI 3.2 kb fragment. The background bands for this gel is much higher than in Figure 11. This is because the probe DNA is homologous to the repeated sequences shown underlined in Figure 10 as well as other repeated sequences within this region as discussed above. In lanes 1-3, the DNA is cut with Bam HI. The expected pattern of hybridization is a 9.05 kb band in the lanes with wild type DNA. An additional 10.25 kb band is seen in the lane with \textit{su(f)^{hd252}} DNA demonstrating the insertion of the 1.2 kb P-element. The insertion of this element is demonstrated again in lanes 4-6. The DNA is digested with Bam HI and Pst I. The wild type pattern shows a 7.4 kb band while the pattern in the lane with \textit{su(f)^{hd252}} DNA shows an additional 8.6 kb band. The additional bands, such as the 4.5 kb band, are due to cross homology of the probe. In lanes 7-9, the DNA is digested with Hind III. The wild type pattern of hybridization shows a 5.4 kb band which is the fragment from the Hind III site at -3.2 to the Hind III site at +2.2. If the P-element Hind III site at 0.0 is the only one present, and the P-element is 1.2 kb, then this digestion should yield a band of approximately 4.4 kb size. However, the additional band seen in the \textit{su(f)^{hd252}} DNA in lane 9 is 3.6 kb. This shows that a second Hind III site must be present within the P-element at approximately P-element Hind III (0.9). This fragment would be from the -3.2 Hind III site to +0.1 (the insertion site) plus 0.3 kb into the element. When the DNA is cut with Bam HI and Hind III as in lanes 10-12, the same 5.4 kb fragment is expected to hybridize in the wild type lanes and consequently the same additional 3.6 kb fragment is seen in the \textit{su(f)^{hd252}}
Figure 12. Restriction Fragment Length Polymorphism Analysis on the $su(f)^{hd252}$ allele using the Eco R I 3.2 kb Probe

Lanes 1, 4, 7, 10, 13, and 16 contain $y^2\,wa\,f^I\cdot y^+ / FM7$ DNA

Lanes 2, 5, 8, 11, 14, and 17 contain $FM7 / Y$ DNA

Lanes 3, 6, 9, 12, 15, and 18 contain $y^2\,wa\,f^I\,su(f)^{hd252} / FM7$ DNA
DNA lane. In lanes 13-15, the DNA is cut with Xho I and Bam HI. A 6.8 kb band hybridizes in the wild type DNA lanes while an additional 5.35 kb band hybridizes in the lane with su(f)^{hd252} DNA. Finally in lanes 16-18, the DNA is cut with Xho I and Pst I yielding a 9.5 kb band in the lanes with wild type DNA and an additional 8.05 kb band in the lane with su(f)^{hd252} DNA. The RFLPs in lanes 15 and 18 again show the presence of the P-element Xho I (0.75) site. The smaller bands hybridizing in these lanes are also due to the cross homology of the probe.

The P-element insertion into the su(f) gene has resulted in the su(f)^{hd252} allele. The element is inserted at a map location of +0.1 (Figure 10). Its restriction pattern includes a Hind III site at its proximal border, 0.0. It also contains an Xho I site 0.75 kb distal to the Hind III site and another Hind III site 0.9 kb distal to the Hind III site at 0.0.

The other su(f) alleles in which RFLPs were found were su(f)^{94} and su(f)^{100}, as mentioned earlier. The isolation and characterization of these alleles, as well as the su(f)^{R617} allele, was discussed previously in the Genetic Results section. The RFLPs associated with the su(f)^{94} and su(f)^{100} alleles was detected in the original screen for RFLPs. Figures 13 and 14 represent the repeated experiments in order to thoroughly document the RFLP. The su(f)^{R617} allele was included on this repeated experiment for two reasons. The first was that its DNA had never been subjected to molecular analysis, and that its initial phenotype was very similar to the phenotype of the su(f)^{94} and su(f)^{100} alleles.

In both Figures 13 and 14 the DNA isolated was from genotypes of homozygous y^2 w^a f^l \cdot y^+, y^2 w^a f^l su(f)^{94}, y^2 w^a f^l su(f)^{100}, and y^2
It was restricted with the enzymes indicated and organized in groups of four; that is, in lane 1 is $y^2 w^a f^l \cdot y^+$, in lane 2 is $y^2 w^a f^l su(f)^{94}$, in lane 3 is $y^2 w^a f^l su(f)^{100}$, and in lane 4 is $y^2 w^a f^l su(f)^R617$. This sequence of genotypes is then repeated in lanes 5-8, 9-12, and 13-16. The probe used in Figure 13 is the Xho I 4.4 kb fragment. The background hybridization for this gel is much higher than in Figure 14. This is again because the probe DNA is homologous to the repeated sequences shown underlined in Figure 10 as well as other repeated sequences within this region as discussed above. The DNA in lanes 1-4 was digested with Eco RI. No difference in band size was detected between the DNA of wild type versus that of the $su(f)$ alleles. The smaller hybridizing bands in these lanes are due to the cross homology of the probe. In lanes 5-8, the DNA was restricted with Bam HI. The pattern of hybridization is a strong 9.0 kb band in the lane with wild type DNA. The expected hybridization pattern would be a strong 9.0 kb band. The Xho I 4.4 kb probe hybridizes almost equally to the fragment from the Bam HI site at +13.25 to the Bam HI site at +4.25 and to the fragment from the Bam HI site at +4.25 to the Bam HI site at -4.8. In the lanes containing the $su(f)^{94}$ and $su(f)^{100}$ DNA, a doublet of bands hybridizes. One is approximately 9.0 kb and the other 7.0 kb. Only a single, strong 9.0 kb band hybridizes in the lane with $su(f)^R617$ DNA. This difference is due to an additional Bam HI site at +11.15 as indicated on the molecular map (Figure 10). The RFLP for the $su(f)^{94}$ allele is the same as the $su(f)^{100}$ allele. This finding led to an in-depth genetic study of these alleles and the hypothesis about their origin as discussed in the Genetic Results. In lanes 9-12, the DNA
Figure 13. Restriction Fragment Length Polymorphism

Analysis on the $su(f)^{94}$, $su(f)^{100}$, and $su(f)^{R617}$ alleles using the Xho I 4.4 kb Probe

Lanes 1, 5, 9, and 13 contain $y^2 \wa f^1 \cdot y^+ / y^2 \wa f^1 \cdot y^+$ DNA

Lanes 2, 6, 10, and 14 contain $y^2 \wa f^1 \; su(f)^{94} / y^2 \wa f^1 \; su(f)^{94}$ DNA

Lanes 3, 7, 11, and 15 contain $y^2 \wa f^1 \; su(f)^{100} / y^2 \wa f^1 \; su(f)^{100}$ DNA

Lanes 4, 8, 12, and 16 contain $y^2 \wa f^1 \; su(f)^{R617} / y^2 \wa f^1 \; su(f)^{R617}$ DNA
was digested with Bam HI and Eco RI. In the lane with wild type DNA, a 7.55 kb band and a 4.45 kb band hybridizes. These are the fragments from the Eco RI site at +11.18 to the Bam HI site at +4.25 and the fragment from the Bam HI at +4.25 to the Eco RI site at -0.2, respectively. The hybridization pattern in the lanes with the \( su(f)^{94} \) and \( su(f)^{100} \) DNA shows a 6.9 kb band and the same 4.45 kb band. This difference is due to the presence of the additional Bam HI site at +11.15. When the \( su(f)^{R617} \) DNA is compared to wild type DNA for this digestion, no differences are detected. Finally, in lanes 13-16, the DNA was digested with Sal I. No differences are detected for the DNA from the \( su(f) \) alleles when compared to the wild type DNA. Lanes 1-4 and 13-16 provide evidence that the RFLP is not due to a small insertion or deletion at the +11.15 location since there are both an Eco RI and a Sal I site spanning the site.

The main purpose of the results presented in Figure 14 is to demonstrate that the additional Bam HI site is indeed associated with the more proximal 9.0 kb Bam HI fragment and not located 7.0 kb distal to the +4.25 Bam HI fragment. Since the X-H 1.9 kb probe would not normally hybridize to the more proximal Bam HI 9.0 kb fragment, it was the probe used in Figure 14, a blot identical to the one in Figure 13. The DNA was digested with Eco RI in lanes 1-4, with Bam HI in lanes 5-8, with Bam HI and Eco RI in lanes 9-12, and with Sal I in lanes 13-16. No differences are detected among any of the alleles when compared to wild type. This clearly demonstrates that the RFLP is associated with the region proximal to the +4.25 Bam HI site not the more distal region covered by the X-H 1.9 kb probe. In conclusion, the RFLP is due to an additional Bam HI site
Figure 14. Restriction Fragment Length Polymorphism

Analysis on the $su(f)^{94}$, $su(f)^{100}$, and $su(f)^{R617}$ alleles using the Xho I- Hind III 1.9 kb Probe

Lanes 1, 5, 9, and 13 contain
$y^2 \text{wa} f^1 \cdot y^+ / y^2 \text{wa} f^1 \cdot y^+$ DNA

Lanes 2, 6, 10, and 14 contain
$y^2 \text{wa} f^1 \ su(f)^{94} / y^2 \text{wa} f^1 \ su(f)^{94}$ DNA

Lanes 3, 7, 11, and 15 contain
$y^2 \text{wa} f^1 \ su(f)^{100} / y^2 \text{wa} f^1 \ su(f)^{100}$ DNA

Lanes 4, 8, 12, and 16 contain
$y^2 \text{wa} f^1 \ su(f)^{R617} / y^2 \text{wa} f^1 \ su(f)^{R617}$ DNA
located at +11.15 and is only associated with the \( su(f)^{94} \) and \( su(f)^{100} \) alleles. The hypothesis about the origin of these mutations and their identical molecular lesions, is presented in the results of the Complementations Studies in the Genetic Results Section.
CONCLUSIONS

The goal of this work was to gain a better understanding of the gene structure, pattern of expression, and complicated mechanics of action of the su(f) gene and much of this has been accomplished. Many questions presented in the introduction of this study have been answered while others remain unanswered. One of the key questions addressed was the genetic separability of suppression and enhancement in the su(f) locus. The answer to the question was largely provided by the complementation studies. In these studies, the numerical assessment of the alleles produced a more objective phenotypic description of both the heterozygous and the homozygous flies than has been reported before. The results of these studies strongly support the model of action by demonstrating very important findings. The su(f) alleles demonstrate a variety of suppression and enhancement phenotypes. Some su(f) alleles are strong enhancers of \( w^a \) and strong suppressors of \( f \), some are weak enhancers of \( w^a \) and strong suppressors of \( f \), some are strong enhancers of \( w^a \) and weak suppressors of \( f \), while some are weak enhancers of \( w^a \) and weak suppressors of \( f \). This variety of phenotypes shows that the suppression and enhancement effects do not weaken or strengthen in concert. In fact, the suppression and enhancement effects are independent of one another and the phenotype is a result of how the su(f) allele independently modifies the target allele. Several different su(f) alleles show a stronger enhancement of the \( w^a \) phenotype at low temperatures than at high temperatures. The alleles also demonstrate a weaker suppression of the \( f \) phenotype at the low
temperatures and stronger one at the high temperatures. The main point is that if this suppression / enhancement ability of the su(f) product were a singular one, how could it affect the transcription at two different loci at the same time and respond to temperature shifts in an entirely opposite ways? This finding, not only from these complementation studies, but from those completed prior to this study as described in the Introduction, have been essential in the formulation of the current model of action. These results indicate that indeed the suppression and enhancement effects of the su(f) alleles are genetically separable events and consequently represent two different functional domains probably within the same protein product. Other explanations for this pattern might be variable splicing which results in two protein products, or even tissue specific action of the su(f) protein.

Many of the published reports on the phenotype of su(f) and its target loci are based on one allele of su(f), su(f)\(^1\). As discussed in the Results section, this allele demonstrates very strong enhancement and very strong suppression effects. This allele is not, however, representative of the wide range of suppression / enhancement phenotypes seen in most of the su(f) alleles. The genetic separability of suppression and enhancement is an important part of the current model of action and is an important result in terms of general knowledge about the su(f) locus.

Another important result came from the phenotypic studies done on the su(f) alleles in cis with the \(lz\) alleles. First of all these results support those presented in the literature, that \(lz^1\) is suppressed, \(lz^{37}\) is enhanced, and \(lz^{34}\) is not affected, in cis with the su(f)\(^1\) allele. However, this type of analysis
has never been reported on the su(f)ts678, su(f)94, su(f)ts726, nor su(f)R918 alleles. The results with the IzI allele and the Iz34 allele show that the su(f)ts678 and su(f)94 alleles are weaker suppressor alleles than su(f)I and are expected based on their respective interactions with forked. The enhancement phenotype was shown only when su(f)I interacted with Iz37. The suppression phenotypes of IzI with the su(f) alleles were predicted since the IzI allele is due to an insertion of a gypsy element. Both Iz37 and Iz34 are spontaneous alleles of Iz, but the type of spontaneous mutation is not known. Perhaps after the Iz locus is cloned and the alleles closely examined the explanation for these phenotypes can be explained (Mel Green, personal communication). These findings go a step further in demonstrating the separability of suppression and enhancement. Each su(f) allele affects a Iz allele in only one way, a suppressed phenotype or an enhanced phenotype. Only one of these domains can be active in order to achieve these phenotypic results. A result that was seen, which was predicted by the model, was that the weak alleles of su(f) weakly modified the Iz alleles while strong su(f) alleles greatly modified the Iz alleles. Again these findings show that the ability to suppress a phenotype and enhance a phenotype are genetically separable.

Tests completed on the two new su(f) alleles, su(f)94 and su(f)100, as well as the su(f) reversion allele, su(f)R617, allowed for the examination of these phenotypes in new genetic backgrounds by recombining away target loci alleles then crossing on new target loci alleles. These new alleles of su(f), are now categorized as "weak" alleles. This is because their suppression and enhancement effects are so mild. This finding alone is a
strong statement about the *su(f)* locus. Even in an allele that shows such a weak phenotype, a suppression / enhancement phenotype is seen nonetheless. This leads us to conclude that the *su(f)* product acts without a minimal threshold level. It functions as a regulator of transposable transcription regardless of the level of product produced.

The goal of making new mutants with detectable RFLPs was completed, resulting in *su(f)*^94^ and *su(f)*^100^. However, neither the construction of an intragenic deletion nor a marked P element insertion mutation was completed. The two new *su(f)* alleles, as well as 18 other *su(f)* alleles, were subjected to extensive molecular analyses. Many of these alleles are unique to this research group. A new genomic map was constructed and a region of approximately 26.5 kb was mapped. Each allele was examined with seven different restriction enzymes and 4 different probes. Among all these alleles only 2 new RFLPs were discovered and were associated with the two new *su(f)* alleles, as stated above. These two were in addition to the RFLPs found with the *su(f)*^hd252^ allele which was due to the insertion of a P element. These new RFLPs were located on the genomic map of *su(f)*. The RFLPs are located outside the "region of transcription" as defined by K. O'Hare (Lindsley and Zimm, 1990). They might be located in a part of the gene that has not been fully molecularly characterized or they could be a mutation that is not the cause of the resulting phenotype. However, the goal of determining the molecular lesion of any or all of these 20 alleles was accomplished. The next goal was to correlate these molecular lesions with the phenotype of the allele in hopes of further understanding the physical nature of the *su(f)* locus and its fine structure.
map. Although this goal was not accomplished and the answers not obtained with this method, the possibility of obtaining these types of results still exists. Other methods such as transcriptional analyses and especially sequencing of the locus must be employed to define the physical nature of this locus.

These two sets of planned experiments, the mutagenesis experiments and the RFLP studies, were not large enough in the size and scope in order to fully answer these questions. The mutagenesis experiments overall did produce two new weak \( su(f) \) alleles with detectable molecular lesions. It had been hoped that additional mutants could be obtained, but upon reviewing mutagenesis procedures from the literature as well as those conducted in this same research group, the size of the experiments was simply too small to expect many positive results. For example, out of 23 \( su(s) \) mutations obtained in five different mutagenesis screens employing 4 different mutagens, only two showed detectable molecular lesions (Voelker et al., 1989). Many RFLP studies in \textit{Drosophila} such as this one, do not result in large numbers of alleles with detectable molecular lesions.

Finally, the \textit{in situ} hybridization experiments showed that the cloned \( su(f) \) DNA did indeed hybridize to the predicted salivary band locations corresponding to \( su(f)^+ \). The \textit{in situ} hybridization of the cloned \( su(f) \) DNA to salivary gland chromosomes has not previously presented. This portion of the study concludes that these cloned fragments do hybridize to the correct regions corresponding to the \( su(f) \) locus. Therefore, the hybridization of the cloned fragments used as probes in this experiment as well as the RFLP study is unique to the \( su(f) \) region.
The model presented in Figure 1 is a good hypothesis for the \textit{su(f)}+ action. As a whole, it was supported by this research. The genetic separation of the suppression and enhancement functions were proven by the complementation studies and single target gene studies. Many more experiments need to be completed in order to answer the question of the gene's effect on lethality and the blockage of \textit{glue protein} transcription and thus the ecdysterone transcription. The physical limits and molecular structure of this gene need to be established in order to fully understand its genetic actions and phenotypes.

There are many experiments that remain to be completed in order to more fully understand the \textit{su(f)} locus. One is to molecularly analyze the transcription patterns of the many \textit{su(f)} alleles. An example of just such an effort was reported in the literature by Markopoulou et al., 1989. Northern analysis was used to examine whether \textit{Notch} transcription was affected in the \textit{facet} alleles. This same type of analysis would help the investigator learn if mutations at \textit{su(f)} are due to splicing differences, transcriptional termination, positional effect, promoter and enhancer functions, or various transcriptional defects. These types of answers can be obtained by this type of study since the presence or absence of a physical lesion within the DNA does not always correlate to the transcriptional pattern of the gene.

The most pressing information needed to further the understanding of \textit{su(f)} would be to understand the \textit{su(f)} protein products. Since the product is so intimately involved in trans-acting gene regulation, the knowledge of what the product is and how it works, would go a great distance in helping
to understand the function of many suppressor loci. Questions such as whether or not su(f) produces one, two, or more protein products and where these products are localized throughout development are essential. Does su(f) interact directly with the target loci DNA or indirectly bind to an intermediate DNA thus causing the suppression and enhancement effects as well as prevent the lethal phenotypes? The practical side of this research would involve obtaining all the genomic clones and finalizing the su(f) cDNA complement. These cDNA sequences would need to be sequenced in order to obtain a putative protein product. These sequences could be compared to other known DNA binding products as well as compared to the sequences of other Drosophila suppressor loci products when that data becomes available.

Many important questions can be better understood from these types of studies. The phenomena of suppression in Drosophila and the mechanisms of its action can be elucidated. Why do genes in Drosophila act solely (or seemingly so) to regulate the transcription of transposable elements? What true benefit does this regulation offer the individual organism as well as the species? Did genes such as these suppressor loci evolve to regulate transposable element transcription or was the already intact mechanism of trans-acting gene regulation altered to accommodate the need for regulating the transcription of transposable elements? These questions are applicable, not only to Drosophila, where many species of transposable elements are known, but to every system which contains transposable elements which are thus transcriptionally regulated.
By studying the phenomena of suppression, the general knowledge of gene regulation as a whole can be advanced. Many suppressor systems do not seem to be involved in a natural descending order of transcriptional gene regulation found in the normal developmental process. They, instead, seem to be situations of gene regulation that arose out of necessity. The resulting phenotypes of suppressor systems are often quite obvious and thus genetically identifiable. If the gene regulation involved in the suppressor systems can be better understood in many of the experimental organisms, then mechanisms of gene regulation will be better understood overall.


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ACKNOWLEDGEMENTS

I would like to thank Dr. Jack Girton for his help and guidance as a major professor. He has been extremely helpful in his assistance during this writing process and throughout my graduate education. I have learned a great deal from him and hope that with his training I am only beginning my scientific education.

I would like to thank Dr. Bill Welshons for his patient teaching throughout the years. He has been not only an inspiration as a scientist, but a friend as well.

I would like to thank Dr. Lois Girton for her helpful assistance while I was learning many lab techniques. Also I would like to thank her for the unpublished results presented in this thesis.

I would like to thank Jean Welshons, Karen Duus, and Ginny Lephardt for their excellence in maintaining an organized lab. Their help went beyond measure as they were always willing to pitch in when I needed them. Their efficiency made the task of Drosophila genetics that much easier. I would also like to thank Franchesca Winandy for her help in preparing salivary gland chromosome squashes.

I would like to thank Maureen Gorman for her friendship and support as a fellow graduate student; it was a necessity.

Finally I would like to thank my husband, Andy, for his patience understanding, and love, during these graduate school years. Without him, I would not have made it through. Andy- I promise, I won't get another Ph.D.!
APPENDIX A
Table A-1. Results of the first round of TEM mutagenesis

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* Nonvirgin parent present resulting in incorrect progeny, the bottle was thrown out after the initial screening.
Table A-2. Results of the second round of TEM mutagenesis

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<td>69</td>
<td>78</td>
<td>83</td>
<td>75</td>
<td>50</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4X</td>
<td>76</td>
<td>76</td>
<td>143</td>
<td>118</td>
<td>78</td>
<td>88</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4X</td>
<td>113</td>
<td>111</td>
<td>89</td>
<td>80</td>
<td>79</td>
<td>97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2X</td>
<td>58</td>
<td>65</td>
<td>128</td>
<td>98</td>
<td>63</td>
<td>76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2X</td>
<td>79</td>
<td>91</td>
<td>115</td>
<td>83</td>
<td>79</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| TEM Total | 1751 + 2527 = 4278 |

$^a$ Nonvirgin parent present resulting in incorrect progeny, the bottle was thrown out after the initial screening.
Table A-3. Results of the G₂ cross determining potential *su(f)* mutants

<table>
<thead>
<tr>
<th>G₂ Cross</th>
<th>Expected Progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potentials</td>
<td>y²w⁺ + f¹ * y⁺</td>
</tr>
<tr>
<td>y²w⁺ct⁶ f¹ DfVE738</td>
<td>B⁵Y</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>11</td>
<td>20</td>
</tr>
</tbody>
</table>

ᵃ Males were mated to *su(f) 1 / su(f) 1* virgins and screened as heterozygous females in G₃.
Table A-4. Results of the "No DEB" Control Cross

<table>
<thead>
<tr>
<th>No DEB Control</th>
<th>♀</th>
<th>♂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross 1 Bottle 1a</td>
<td>75</td>
<td>76</td>
</tr>
<tr>
<td>Cross 1 Bottle 2a</td>
<td>62</td>
<td>78</td>
</tr>
<tr>
<td>Cross 1 Bottle 1b</td>
<td>55</td>
<td>42</td>
</tr>
<tr>
<td>Cross 1 Bottle 2b</td>
<td>56</td>
<td>64</td>
</tr>
<tr>
<td><strong>Subtotal Cross 1</strong></td>
<td><strong>266</strong></td>
<td><strong>262</strong></td>
</tr>
<tr>
<td>Cross 2 Bottle 1a</td>
<td>73</td>
<td>98</td>
</tr>
<tr>
<td>Cross 2 Bottle 2a</td>
<td>71</td>
<td>65</td>
</tr>
<tr>
<td>Cross 2 Bottle 1b</td>
<td>122</td>
<td>114</td>
</tr>
<tr>
<td>Cross 2 Bottle 2b</td>
<td>107</td>
<td>106</td>
</tr>
<tr>
<td><strong>Subtotal Cross 2</strong></td>
<td><strong>373</strong></td>
<td><strong>363</strong></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>641</strong></td>
<td><strong>645</strong></td>
</tr>
</tbody>
</table>
### Table A-5. Results of the Control Cross determining the number of lethal mutational lesions on the X chromosome due to DEB

<table>
<thead>
<tr>
<th>Control Cross</th>
<th>♂</th>
<th>♀</th>
<th>♀♀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross 1 Bottle 1a</td>
<td>61</td>
<td>71</td>
<td>0</td>
</tr>
<tr>
<td>Cross 1 Bottle 2a</td>
<td>103</td>
<td>93</td>
<td>1</td>
</tr>
<tr>
<td>Cross 1 Bottle 1b</td>
<td>52</td>
<td>73</td>
<td>2</td>
</tr>
<tr>
<td>Cross 1 Bottle 2b</td>
<td>69</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td><strong>Subtotal Cross 1</strong></td>
<td>305</td>
<td>307</td>
<td>%♂♀ 99.3</td>
</tr>
<tr>
<td>Cross 2 Bottle 1a</td>
<td>61</td>
<td>111</td>
<td>2</td>
</tr>
<tr>
<td>Cross 2 Bottle 2a</td>
<td>59</td>
<td>91</td>
<td>1</td>
</tr>
<tr>
<td>Cross 2 Bottle 1b</td>
<td>137</td>
<td>140</td>
<td>0</td>
</tr>
<tr>
<td>Cross 2 Bottle 2b</td>
<td>120</td>
<td>125</td>
<td>1</td>
</tr>
<tr>
<td><strong>Subtotal Cross 2</strong></td>
<td>377</td>
<td>467</td>
<td>%♂♀ 80.7</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>682</td>
<td>774</td>
<td>%♂♀ 88.1</td>
</tr>
</tbody>
</table>
Table A-6. DEB induced Potential Mutations - G2 screen Results

DEB Potentials Score Sheet

<table>
<thead>
<tr>
<th>Potential #</th>
<th>Origin</th>
<th>Expected Progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.2 1.1 2.2 1.1 2.2 1.1 2.2 1.1 2.2 1.1</td>
</tr>
<tr>
<td>1</td>
<td>V- Cross 1 Bottle 13a Day E</td>
<td>33 16 33 20 X X X X</td>
</tr>
<tr>
<td>2</td>
<td>V- Cross 1 Bottle 23b Day E</td>
<td>22 12 21 12 X X X X</td>
</tr>
<tr>
<td>3a</td>
<td>NV- Cross1 Bottle 23b Day E</td>
<td>(23) 7 (27) 7 (23) 13 (27) 18</td>
</tr>
<tr>
<td>3b</td>
<td>NV- Cross1 Bottle 23b Day E</td>
<td>(23) 12 (16) 12 (23) 4 (16) 1</td>
</tr>
<tr>
<td>4a</td>
<td>NV- Cross1 Bottle 24b Day E</td>
<td>(24) 18 (26) 22 (24) 3 (26) 1</td>
</tr>
<tr>
<td>4b</td>
<td>NV- Cross1 Bottle 24b Day E</td>
<td>21 22 22 17 0 0 0 0</td>
</tr>
<tr>
<td>5a&amp;b</td>
<td>NV- Cross1 Bottle 24b Day E</td>
<td>50 41 47 54 0 0 0 0</td>
</tr>
<tr>
<td>6a&amp;b</td>
<td>NV- Cross1 Bottle 31b Day E</td>
<td>61 41 55 36 0 0 0 0</td>
</tr>
<tr>
<td>7a&amp;b</td>
<td>NV- Cross1 Bottle 32b Day E</td>
<td>45 33 62 37 0 0 0 0</td>
</tr>
<tr>
<td>8</td>
<td>V- Cross 2 Bottle 5a Day 8</td>
<td>21 20 23 16 X X X X</td>
</tr>
<tr>
<td>9a&amp;b</td>
<td>NV- Cross2 Bottle 9a Day 8</td>
<td>36 43 40 58* 0 0 0 0</td>
</tr>
<tr>
<td>10</td>
<td>V- Cross 2 Bottle 13a Day 8</td>
<td>21 15 24 14 X X X X</td>
</tr>
<tr>
<td></td>
<td>NV- Cross2 Bottle18a Day 8</td>
<td></td>
</tr>
<tr>
<td>11a</td>
<td>(34)</td>
<td>11</td>
</tr>
<tr>
<td>11b</td>
<td>(34)</td>
<td>31</td>
</tr>
<tr>
<td>12a</td>
<td>(34)</td>
<td>25</td>
</tr>
<tr>
<td>12b</td>
<td>(34)</td>
<td>38</td>
</tr>
<tr>
<td>13</td>
<td>V- Cross 2 Bottle 23a Day 8</td>
<td>18</td>
</tr>
<tr>
<td>14</td>
<td>V- Cross 2 Bottle 25a Day 8</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>V- Cross 2 Bottle 30a Day 8</td>
<td>20</td>
</tr>
<tr>
<td>16</td>
<td>V- Cross 2 Bottle 32a Day 8</td>
<td>5</td>
</tr>
<tr>
<td>17a</td>
<td>NV- Cross2 Bottle 6b Day 5</td>
<td>(37)</td>
</tr>
<tr>
<td>17b</td>
<td>NV- Cross2 Bottle 6b Day 5</td>
<td>(27)</td>
</tr>
<tr>
<td>18a &amp; b</td>
<td>NV- Cross2 Bottle 9b Day 5</td>
<td>65</td>
</tr>
<tr>
<td>19</td>
<td>V- Cross 2 Bottle 14b Day 5</td>
<td>19</td>
</tr>
<tr>
<td>20a</td>
<td>NV- Cross2 Bottle 21b Day 5</td>
<td>(30)</td>
</tr>
<tr>
<td>20b</td>
<td>NV- Cross2 Bottle 21b Day 5</td>
<td>(32)</td>
</tr>
<tr>
<td>21a</td>
<td>NV- Cross2 Bottle 11b Day 7</td>
<td>20</td>
</tr>
<tr>
<td>21b</td>
<td>NV- Cross2 Bottle 11b Day 7</td>
<td>28</td>
</tr>
<tr>
<td>22a</td>
<td>NV- Cross2 Bottle 31b Day 7</td>
<td>18</td>
</tr>
<tr>
<td>22b</td>
<td>NV- Cross2 Bottle 31b Day 7</td>
<td>40</td>
</tr>
<tr>
<td>23</td>
<td>V- Cross 2 Bottle 11a Day 8</td>
<td>20</td>
</tr>
<tr>
<td>24</td>
<td>V- Cross 2 Bottle 30a Day 8</td>
<td>17</td>
</tr>
<tr>
<td>25</td>
<td>V- Cross 2 Bottle 11b Day 8</td>
<td>-</td>
</tr>
<tr>
<td>26</td>
<td>V- Cross 2 Bottle 23b Day 8</td>
<td>21</td>
</tr>
<tr>
<td>27</td>
<td>V- Cross 2 Bottle 25b Day 8</td>
<td>6</td>
</tr>
<tr>
<td>28</td>
<td>V- Cross 2 Bottle 26b Day 8</td>
<td>22</td>
</tr>
<tr>
<td>29</td>
<td>V- Cross 2 Bottle 31b Day 8</td>
<td>12</td>
</tr>
<tr>
<td>30</td>
<td>M- Mosaic from F2 screen</td>
<td>157</td>
</tr>
</tbody>
</table>
APPENDIX B
Figure B-1. Single P element mutagenesis schematic for the crosses involved
Single P element mutagenesis of su(f)

\[ G_0 \quad \frac{y^2 \text{wa Pftz-βgal} f^1}{y^2 \text{wa Pftz-βgal} f^1} : \text{TM6,Tby} \quad X \quad \frac{y^2 \text{wa f}^1}{y^2 \text{wa f}^1} : \frac{\text{ry506 wc(ry^*)}}{\text{TM3,Sb}} \]

Select for Dysgenic females
(for Tby and against Sb)

\[ G_1 \quad \frac{\frac{y^2 \text{wa Pftz-βgal} f^1}{y^2 \text{wa Pftz-βgal} f^1}}{\frac{\text{ry506 wc(ry^*)}}{\text{TM6,Tby}}} \quad X \quad \frac{\frac{y^2 \text{wa f}^1}{y^2 \text{wa f}^1}}{\frac{\text{su(f)}^1 \cdot y^+}{\text{ry506}}} \]

or

\[ \frac{\frac{y^2 \text{wa f}^1}{y^2 \text{wa f}^1}}{(*)} : \frac{\text{ry506 wc(ry^*)}}{\text{ry506}} \quad \frac{\frac{y^2 \text{wa f}^1}{y^2 \text{wa f}^1}}{(*)} : \frac{\text{TM6,Tby}}{\text{ry506}} \]

non Tby females
-alternative way to screen potentials
continued-

Select for Tby females

\[ G_2 \quad \frac{\frac{y^2 \text{wa f}^1}{y^2 \text{wa f}^1}}{(*)} : \frac{\text{TM6,Tby}}{\text{FM7, +}} \quad X \quad \frac{\text{FM7}}{\text{+}} \]

Screen potential mutants

Select against y+ and for Tby

\[ G_3 \quad \frac{\frac{y^2 \text{wa f}^1}{y^2 \text{wa f}^1}}{(*)} : \frac{\text{TM6,Tby}}{\text{FM7}} \quad \frac{\text{FM7}}{\text{+}} \]

[Look at (*) / Y males for lethal or mutant phenotype.
"2nd generation screen"]

\[ G_4 \quad \frac{\frac{y^2 \text{wa f}^1}{y^2 \text{wa f}^1}}{(*)} : \frac{\text{FM7}}{\text{BSy}} \quad \text{Stock} \]
Alternative Method of Screening Potentials

\[ \text{non Tby females} \]
\[ \frac{y^2 w^a f^1 (\ast)}{y^2 w^a f^1 su(f)^1 \cdot y^+} \]
\[ \frac{ry^{506} wc(ry^+)}{ry^{506}} \]

\[ \rightarrow \]
\[ \frac{y^2 w^a f^1 (\ast)}{y^2 w^a f^1 su(f)^1 \cdot y^+} \]
\[ \frac{ry^{506} wc(ry^+)}{ry^{506}} \]

\[ \downarrow \]

\[ G_2 \]
\[ \frac{y^2 w^a f^1 (\ast)}{y^2 w^a f^1 su(f)^1 \cdot y^+} \]
\[ \frac{ry^{506} wc(ry^+)}{ry^{506}} \]

\[ \times \]
\[ \frac{y^2 + f^1 su(f)^1 \cdot y^+}{ry^{506}} \]

\[ \frac{ry^{506}}{ry^{506}} \]

Select for \( y^+ \) ry^{506} and Ubx

\[ G_3 \]
\[ \frac{y^2 w^a f^1 (\ast)}{y^2 + f^1 su(f)^1 \cdot y^+} \]
\[ \frac{ry^{506}}{ry^{506}} \]

\[ \times \]
\[ \frac{FM7 \cdot TM2 \cdot Ubx}{+} \]

Screen potential mutants

\[ G_4 \]
\[ \frac{y^2 w^a f^1 (\ast)}{FM7} \]
\[ \frac{TM2 \cdot Ubx}{ry^{506}} \]

\[ \times \]
\[ \frac{FM7}{+} \]

Select for Ubx

\[ G_5 \]
\[ \frac{y^2 w^a f^1 (\ast)}{FM7} \]
\[ \frac{TM2 \cdot Ubx}{ry^{506}} \]

\[ \times \]
\[ \frac{FM7}{+} \]

Select against Ubx

\[ G_6 \]
\[ \frac{y^2 w^a f^1 (\ast)}{FM7} \]
\[ + \]

\[ \frac{FM7}{B^S Y} + \text{Stock} \]

Note:
\[ \frac{y^2 w^a f^1 su(f)^1 \cdot y^+}{ry^{506}} \]
\[ \times \]
\[ \frac{FM7 \cdot TM2 \cdot Ubx}{+} \]

In the alternative way to pick up mutants 50% of the females are \( y^+ \), ry^{506}, and Ubx will be the above genotype and will appear to show the su(f) phenotype, thus giving false positive mutants. Therefore by selecting against \( y^+ \) in G4 it is possible to eliminate this problem.

\[ \text{(All females will be } y^+ \text{ and will be selected against in } G_4) \]