EMS AND X-RAY INDUCED MUTABILITY IN THE VERMILION REGION OF THE X CHROMOSOME OF DROSOPHILA MELANOGASTER

A thesis submitted in partial satisfaction of the requirements for the degree of Master of Science in Biology

by

Patricia Ann Binney

September, 1970
The thesis of Patricia Ann Binney is approved:

Committee Chairman

San Fernando Valley State College
September, 1970

ii
ACKNOWLEDGMENTS

The good humored interest and encouragement of Dr. George Lefevre, Jr. made this project a rewarding experience.
# TABLE OF CONTENTS

**LIST OF FIGURES**. .............................................. v

**ABSTRACT**. ................................................ vi

**CHAPTER**

I  **INTRODUCTION** ............................................. 1

II  **REVIEW OF THE LITERATURE** ............................. 3

   The cytology of Section 10
   X-ray-induced breakage
   Female sterility among X-ray-induced
   \( \text{v} \) mutants
   Methods of using duplications
   Complex loci

III  **MATERIALS AND METHODS**. ............................... 11

IV  **RESULTS**. ................................................ 18

   EMS-induced mutations
   2000r-induced mutations
   4000r-induced mutations

V  **DISCUSSION**. ................................................. 25

   Detection of dominant female-sterile
   mutants
   A semi-lethal mutant induced under
   the duplication
   Complex loci
   Mosaic mutants
   Summary

**BIBLIOGRAPHY**. ................................................. 34
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The salivary chromosome cytology of the insertion translocations, T(1;2)v^63i and T(1;2)v^65b, as compared with normal.</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Diagrammatic representation of the duplications and deficiencies used to recover and locate mutants induced in the present experiment.</td>
<td>15</td>
</tr>
</tbody>
</table>
ABSTRACT

EMS AND X-RAY INDUCED MUTABILITY IN THE VERMILION REGION OF THE X CHROMOSOME OF DROSOPHILA MELANOGASTER

by

Patricia Ann Binney

Master of Science in Biology

September, 1970

A study was made of EMS and X-ray-induced mutations occurring in the immediate vicinity of the vermillion (v, 1-33.0) locus. This region of the X chromosome was covered by a special Y chromosome containing a duplication extending from salivary chromosome band 9F5 through 10C2, which should permit the recovery of female-sterile or sex-determining mutants in this region. Approximately 6000 F1 males were examined and over 2500 chromosomes tested for the presence of male-lethal and female-sterile mutations; no female-sterile mutants were found. Fourteen recessive lethal mutants were induced, of which 3 were in the covered region. Ten viable and fertile visible mutants, including a lozenge (lz, 1-27.7), a furrowed (fw, 1-36.8), a white (w, 1-1.5), and a garnet (g, 1-44.4)
were also found. The most interesting mutant found was a male-viable \( v \) mutant accompanied by a simultaneously induced, independent semi-lethal mutant located approximately 0.6 crossover units to the right of \( v \).
CHAPTER I
INTRODUCTION

Lefevre (1967), in a study of visible X-ray mutants, found that F₁ females carrying newly induced mutations at the vermilion (v, l-33.0) locus exhibit a high degree of sterility. Approximately 70% of all v mutant F₁ females were sterile after 4000r exposures, but only 8% of yellow (y, l-0.0) and 35% of white (w, l-1.5) mutant F₁ females were sterile. As a result of their sterility, most v mutations are selectively eliminated from the population of mutants normally available for genetic analysis. A cytogenetic analysis of the limited number of fertile X-ray-induced, male-lethal v mutants showed that all of them were deficient for the v locus. Although many extended far to the left, no deficiency extended more than a short distance to the right of the v locus. The above results strongly suggest the existence of a female-sterile locus just to the right of v.

The present experiments were devised specifically to recover such female-sterile mutants following treatment with both ethyl methane sulphonate (EMS) and X rays. In order to eliminate the problem of F₁ female sterility,
the treated X chromosome was maintained in the male line by mating treated males with compound-X females. Since many \(v\) mutants involve male-lethal deficiencies, male viability was insured by providing the male with a special Y chromosome that carries a duplication for the X-chromosome region surrounding the \(v\) locus. Any mutants produced could later be outcrossed, tested for female-sterility and sex-linked recessive lethality, and analyzed cytologically. Although no female sterile mutants were found, a number of mutants of interest were identified. They will be discussed below.
CHAPTER II

REVIEW OF THE LITERATURE

The cytology of Section 10: According to Bridges (1938), Section 10, located in the middle of the euchromatic portion of the X chromosome, contains a total of 63 bands. The vermilion (v, l-33.0) locus is in the dark-staining double band 10A1-2 (Green 1954), and the miniature (m, l-36.1) locus is just to the right of band 10E1-2 (Lindsley and Grell 1968). Besides band 10A1-2, the only other prominent cytological landmark in Section 10 is band 10B1-2. A salivary chromosome preparation of this region is shown in Figure 1. It should be noted that the area between 10A1-2 and 10B1-2 contains only faint bands.

X-ray-induced breakage: Kaufmann (1939, 1946) first studied the response of the various chromosome sections to X-ray-induced breakage. He found that Section 10 showed the lowest coefficient of breakage, based on the number of salivary chromosome bands present. Only 35 of 1048 breaks, or 3.3%, occurring in the euchromatic portion of the X chromosome occurred in Section 10, even though this section contains 6.3% of the total number of bands. Kaufmann, et al. (1948) also noted that Section 10 showed
Figure 1

The salivary chromosome cytology of the insertional translocations, $T(1;2)v^+63i$ and $T(1;2)v^{65b}$, as compared with normal. A) a normal X chromosome showing Sections 9, 10, and 11. The locations of ras, v, m, and $fw$ are indicated. B) $T(1;2)v^+63i$: at the left, the heterozygous deficiency segregant; at the right, the duplication segregant inserted in 2R at 56A and synapsed with the X chromosome. C) $T(1;2)v^{65b}$: at the left, the heterozygous deficiency segregant; at the right, the duplication segregant inserted in the chromocenter. (In both B and C, the limits of the duplications are marked with arrows, and the v locus in 10A1-2 is indicated.) (Lefevre 1969)
little ectopic pairing. Both Hannah (1951) and Kaufmann explained these phenomena in terms of the absence of intercalary heterochromatin in this section.

Since Section 10 seems not to show its fair share of breaks on the basis of salivary chromosome band number, this may not be the proper measure to apply to breakage expectation. Rudkin (1965) found that the distribution of X-ray-induced mutations from interval to interval correlated very well with the relative amounts of DNA present in them. Since Section 10 is made up primarily of faint-staining bands containing little DNA, the low incidence of breakage in this section may be accounted for by a relatively low DNA content (Lefevre 1967).

**Female sterility among X-ray-induced v mutants:**

Lefevre (1967) noted an abnormally high degree of F₁ female sterility among X-ray-induced v mutants. While only 8% of F₁ y mutant females, and 35% of F₁ w mutant females were sterile, 72% of F₁ v mutant females were sterile. Although this sterility could be characteristic of the particular mutant locus involved, in a study of EMS-induced v mutants, Lefevre did not find a high level of sterility of mutant F₁ females. EMS causes a low incidence of cytologically detectable chromosome rearrangements as compared with X rays. A total of 18 female-fertile, X-ray-induced v deficiencies were available for analysis. The most unexpected finding concerning these
deficiencies was that, although the longest extended 48 bands to the left of the v locus, none extended more than 5 or 6 bands to its right. It would appear that the sterile X-ray-induced v mutants are associated with chromosome breakage, most likely deficiency for that part of Section 10A just to the right of the v locus. Such a deficiency, resulting in female sterility, would prevent the recovery of chromosomal mutants in this region.

Two v deficiencies were available for analysis whose right breakpoints fall farther to the right than those of the 18 simple deficiencies mentioned above. Both of these arose in association with an insertional translocation. If they had been simple deficiencies, they would not have been recovered, for in each case the aneuploid deficiency segregant is sterile as a heterozygous female. With the duplicated element present, however, the females are fertile. Df(1)v^{65b} is about 71 bands in length, extending from salivary chromosome band 9F12 or 9F13 through 11A7. Df(1)v^{+63i} is only 34 to 35 bands long, extending from salivary chromosome band 9D4 through 10All. Although the sterility of Df(1)v^{65b} could be accounted for on the basis of its length, Df(1)v^{+63i} is a relatively short deficiency. Four of the female-fertile v deficiencies are longer. When the shorter duplication, Dp v^{+63i}, is added to the longer deficiency, Df(1)v^{65b}, heterozygous females exhibit noticeably
Common to both Df(1)v^{65b} and Df(1)v^{+63i} is a deficiency for bands 9F13 through 10A11. Deficiency for the right portion of this region seems to result in female sterility. Lefevre (1969) suggested that the presence of a female-fertility locus necessary in 2 doses located somewhere between 10A1-2 and 10B1-2 could explain the high level of sterility of X-ray-induced v mutants, the eccentricity of the existing v deficiencies, and the sterility of the translocation deficiency heterozygotes.

This dominant female-sterility effect cannot be attributed to the v locus. Had the 18 analyzed v deficiencies been sterile as F_1 mutant heterozygotes, they would not have been recovered. The fact that none extends farther to the right of the v locus than 10A7 suggests that the sterility locus occupies a position a few bands to the right of v.

The only major deficiency involving Section 10B-F which is available for cytogenetic analysis is Df(1)m^{259-4}. This deficiency, which extends from 10C3 through 10E2 is fertile as a heterozygous female. Therefore, the dominant sterility effect cannot extend as far to the right as 10C3.

Lefevre has been unable to induce v deficiencies that include band 10B1-2. Of the 18 v deficiencies analyzed, 12 had breakpoints through the double band 10A1-2.
Band 10Bl-2 is nearly as large as 10Al-2. Both should contain large amounts of compacted DNA and present sizable targets for breakage. Breaks specifically involving band 10Bl-2 probably have not been detected because of its involvement with, or closeness to, the fertility locus.

Methods of using duplications: Ratty (1954) studied the ability of several autosomally located duplications to "cover" the lethal effects of a series of cytologically known deficiencies involving both the \( w \) and the Notch (\( N, 1-3.0 \)) loci. If a male containing the lethal deficiency is able to survive in the presence of the duplication, the duplication is said to cover the deficiency.

The following procedure was followed in testing the ability of each duplication to cover various deficiencies. Females which were heterozygous for the lethal and a non-lethal balancing inversion were mated to males carrying the duplication. If \( F_1 \) male progeny expressing the phenotype associated with the deficient chromosome carried heterozygously in the female survived, the duplication is able to cover the deficiency. The male carrying the duplication was marked in order to detect nondisjunction.

Covered males were next tested for fertility and to insure they still carried the lethal. If the normal physiological and genetic functions of the mutant chromosome were fully restored by the duplication, 33% of the male progeny should be deficiency males covered by the duplication.
Duplications therefore can be used to protect against the loss of fertility or viability due to induced chromosome mutation.

**Complex loci:** Lewis (1945) studied the dominant mutant Star (\(S\), 2-1.3), which affects eye shape and bristle distribution. Asteroid (\(ast\), 2-1.3), a mutant originally named Star-recessive, is functionally related to Star. \(S^{+}/+ast\) heterozygotes have very small eyes, but \(S^{+}/ast^{+}\) is like \(S/+.\) These two mutants are separable by crossing over, however. \(S\) is located 0.02 units to the left of \(ast\). This is an example of a complex locus involving at least 2 alleles affecting the same phenotype, one dominant, the other recessive. Another example is the dominant mutant Stubble (\(Sb\), 3-58.2) and its recessive allele stubbloid (\(sbd\), 3-58.2). Stubbloid lies approximately 0.01 to 0.03 units to the left of \(Sb\). Welshons (1965) has made extensive studies of the \(N\) locus. Six recessive visible alleles and a number of recessive lethal mutants are known at this complex locus. Notch mutants express both a dominant visible phenotype and a recessive lethal effect.
CHAPTER III
MATERIALS AND METHODS

The following males were used as a source for the \( X \) chromosome to be treated: (1) wild type (+) M56i; (2) yellow-2 (\( y^2 \), 1-0.0) and white-apricot (\( w^a \), 1-1.5); (3) \( y^2 \) chocolate-2 (\( \text{cho}^2 \), 1-5.4).

Female stocks included: (1) A compound double-X stock \([C(1)DX]\), expressing a yellow (\( y \)) and forked (\( f \), 1-56.7) phenotype. \( C(1)DX \) is a reversed acrocentric compound-X chromosome heterozygous for \( \text{In}(1)dl-49 \) and is a useful balancer because of its stability. \( C(1)DX/0 \) is lethal, being homozygous deficient for the bobbed (\( \text{bb} \), 1-66.0) locus. For convenience, this stock is symbolized \( yf: = \). In addition, this stock carried a special \( Y \) chromosome: \( v^+Yy^+ \). This chromosome was produced by irradiating \( B^Sv^+Yy^+ \) (Chovnick 1968) and contains a duplication that extends from 9F5 through 10C1 or 10C2 on Bridges' (1938) revised map of the salivary gland \( X \) chromosome. Our stock contains a mutant \( v \) locus, rather than \( v^+ \), and is symbolized \( vYy^+ \). (2) The second stock, which provided females to which \( F_1 \) males were mated, was \( y^{31d}sc^8f\, dl-49\, v\, w^a \) (yellow-31d, inversion scute 8, \\begin{align*} \text{CHAPTER III} \\
\text{MATERIALS AND METHODS} \\

The following males were used as a source for the \( X \) chromosome to be treated: (1) wild type (+) M56i; (2) yellow-2 (\( y^2 \), 1-0.0) and white-apricot (\( w^a \), 1-1.5); (3) \( y^2 \) chocolate-2 (\( \text{cho}^2 \), 1-5.4).

Female stocks included: (1) A compound double-X stock \([C(1)DX]\), expressing a yellow (\( y \)) and forked (\( f \), 1-56.7) phenotype. \( C(1)DX \) is a reversed acrocentric compound-X chromosome heterozygous for \( \text{In}(1)dl-49 \) and is a useful balancer because of its stability. \( C(1)DX/0 \) is lethal, being homozygous deficient for the bobbed (\( \text{bb} \), 1-66.0) locus. For convenience, this stock is symbolized \( yf: = \). In addition, this stock carried a special \( Y \) chromosome: \( v^+Yy^+ \). This chromosome was produced by irradiating \( B^Sv^+Yy^+ \) (Chovnick 1968) and contains a duplication that extends from 9F5 through 10C1 or 10C2 on Bridges' (1938) revised map of the salivary gland \( X \) chromosome. Our stock contains a mutant \( v \) locus, rather than \( v^+ \), and is symbolized \( vYy^+ \). (2) The second stock, which provided females to which \( F_1 \) males were mated, was \( y^{31d}sc^8f\, dl-49\, v\, w^a \) (yellow-31d, inversion scute 8,
forked, inversion delta-49, vermilion, white-apricot). All of these are sex-linked and fully described by Lindsley and Grell (1968). For convenience, this stock hereafter will be referred to as M-6.

Duplications and deficiencies used in the analysis are described below:

\textbf{Dp(1)v}^{65b}: Obtained from an X-ray-induced insertional translocation. Material deleted from the X chromosome, including bands 9F12 or 9F13 through 11A7, is inserted in the heterochromatic region of chromosome 2. This duplication contains the loci of\( \_v \), miniature (\( \_m, 1\)-36.1), and furrowed (\( \_fw, 1\)-36.8). T(1;2)v\(^{65b} \) males are viable and fertile, show an eye color somewhat lighter than that of wild type, approaching the color of\( \_v \) (presumably because of a position effect on the\( \_v \) locus), and have outstretched wings. The aneuploid duplication segregant is viable, but with reduced fertility, in both sexes. The aneuploid deficiency segregant, as a heterozygous female, is essentially sterile (Lefevre 1969).

\textbf{Dp(1)v}^{+63i}: Obtained from an X-ray-induced insertional translocation. A portion of the X chromosome from 9D4 through 10A11 is inserted in 2R at 56A. This duplication carries both the raspberry (\( \_\text{ras}, 1\)-32.4) and\( \_v \) loci. Duplication-deficiency males are viable, but sterile. Males and females carrying the duplication without the deficiency are viable and fertile. Females heterozygous
for the deficiency are virtually sterile (Lefevre 1969).

Df(1)vL1: X-ray-induced deficiency which extends from 10A1 through 10A5 or 10A6.

Df(1)vL2: X-ray-induced deficiency which extends from 9F12 or 9F13 to 10A1.

Df(1)vL3: X-ray-induced deficiency which extends from 9F7 or 9F8 through 10A7 or 10A8.

Further information concerning the above deficiencies is provided by Lefevre (1969).

Df(1)v64f29: X-ray-induced deficiency which extends from 9F3 or 9F4 through 10A2 (provided by M. M. Green).

Df(1)m259-4: includes bands 10C3 through 10E1-2.

A diagrammatic representation of the above duplications and deficiencies is presented in Figure 2.

Nondeficiency recessive lethal mutants used included:

Lethal l(1)L1: approximately 0.64 crossover units to the right of v.

Lethal l(1)L8: approximately 0.55 crossover units to the right of v.

Lethal l(1)L12: approximately 0.49 crossover units to the right of v.

Male Drosophila less than 24-hours-old, marked with y2 and wa and carrying a normal Y chromosome were fed EMS in a 1% sucrose solution following the method of Lewis
Diagrammatic representation of the duplications and deficiencies used to recover and locate mutants induced in the present experiment.
and Bacher (1968). After feeding, males were mated with
\( y_f: = /v Y y^+ \) females. \( F_1 \) males were screened for vis-
able mutations such as \( v, w, fw, lz, \) etc. Each mutant
male found was mated simultaneously with both \( M-6 \) and
\( y_f: = /v Y y^+ \) females. After 3-4 days of mating, the
two types of females were separated. Males crossed to
\( y_f: = /v Y y^+ \) females constituted the male stock in which
the treated \( X \) chromosome could be perpetuated without
selection. \( F_2 \) females heterozygous for \( M-6 \) and the
treated \( X \) chromosome were used to test for the presence
of either sex-linked recessive lethal or female-sterile
mutants. The \( F_2 \) females were mated with \( M-6 \) males carry-
ing a normal \( Y \) chromosome. The \( F_3 \) was checked for absence
of progeny (female-sterile), or for absence of males with
the treated \( X \) chromosome (sex-linked recessive lethal).
Theoretically only sterile and lethal mutants induced in
the region covered by the \( v Y y^+ \) would be recovered by the
above method. Similar mutants produced elsewhere on the
\( X \) chromosome should die or produce no progeny for analy-
sis.

In addition to the visibly mutant \( F_1 \) males, a number
of duplication-bearing \( F_1 \) males showing no phenotypically
evident mutations were tested for the presence of female-
sterile and lethal mutations.

Visible mutants were analyzed by testing for allel-
isim with known marker stocks. Lethals were tested for
coverage by duplications including \( \text{Dp} \, v^{65b}, \, \text{Dp} \, v^{+63i}, \) and \( v \, Y \, y^+ \). The covered lethals were crossed to the \( v \) deficiencies \( \text{Df}(1)v^{L2} \) and \( \text{Df}(1)v^{L3} \) in an attempt to uncover the lethal locus. To further localize the lethals, crossover data were obtained from the unknown lethal heterozygous with \( 1(1)L1 \).

Two additional experiments were performed following the above procedure, except for the source of the treated \( X \) chromosome. In the first, 7-day-old males marked with \( y^2 \) and \( \text{cho}^2 \) and carrying a normal \( Y \) chromosome were irradiated with 2000r. In the second, 7-day-old wild-type (+) males were irradiated with 4000r.

A supplementary experiment was conducted to test the effect of lowered temperature upon the fertility of \( \text{Df}(1)v^{65b} \) heterozygotes. \( \text{Df}(1)v^{65b} \) heterozygotes with and without \( \text{Dp} \, v^{+63i} \) were mated with \( v \, \text{fw} \) males. Equal numbers of vials were maintained at 18°C and at 25°C. \( \text{Df}(1)v^{65b} \) heterozygotes carrying \( \text{Dp} \, v^{65b} \) were used as controls.
CHAPTER IV

RESULTS

EMS-induced mutations: After feeding with EMS and mating with the C(1)DX females, 41 y^2 w^a males produced a total of 1688 F_1 sons. Of these 1688 males, 92 were phenotypically mutant, but 44 of them were mosaic. Twenty-five of the mosaic mutants were fertile, but all bred wild type (+). Of the 48 nonmosaic mutants, 19 were sterile, 21 bred wild type, and 8 bred true for their original mutant phenotype. These eight mutants included:

(1) a lozenge-like mutant which, when crossed to lz^50e m, showed a slightly rough, but nearly wild type phenotype. This new lz, therefore, complements with lz^50e, as do most lz alleles. (2) a furrowed-like mutant which, when crossed to ras v m f_w, continued to show a marked furrowed phenotype. Thus, the mutant is an allele of f_w. Five of the 8 mutants showed a nearly white eye color in combination with w^a. (3) One of these proved to be allelic with w. This w EMS-induced allele complements with spotted white (w^{SP}). (4) Another of the eye color mutants is allelic to garnet (g, l-44.4). The other three eye color mutants proved more difficult to identify.
since they do not show allelism with any of the eye color mutants available in the laboratory. These mutants were therefore outcrossed to a m f stock in an effort to separate the mutant allele from the w^a allele by crossing over. All of the mutants described above, except for the liz mutant, are viable and fertile as homozygous females.

The eighth and most interesting of the visible mutants found is a v mutant accompanied by an independent, simultaneously-induced, semi-lethal mutant. Males which do hatch are small with narrow abdomens often showing abnormal segmentation. The wings are small, either upswept or incompletely expanded. The eyes are usually small and slightly rough. Genitalia are poorly developed or grossly deformed. Dissection showed these males to be devoid of motile sperm. Many were tested for fertility and all proved to be sterile. The v mutant will be referred to as v^69i, the semi-lethal mutant as s-1(1)69.

This double mutant was subjected to extensive analysis in an attempt to localize s-1(1)69 since it appeared to be closely associated with v^69i, and therefore must be very near to, or within, the region suspected of containing a female-sterile locus. Independent, simultaneously-induced mutants, located only a short distance apart, are rare. These two mutants were both covered by v Y y^+. Although EMS does not usually cause large chromosomal rearrangements, the semi-lethal v mutant was first thought
to be a short deficiency. However, salivary chromosome analysis of the mutant showed it to be cytologically normal.

All 3 of the major duplications used, Dp v65b, Dp v+63i, and v Y y+, covered this lethal. This would place s-1(1)69 somewhere between salivary chromosome bands 9F12 and 10All. Neither Df(1)v12 nor Df(1)vL3 uncovered the lethal, placing it somewhere to the right of 10A6.

Recombination between the y2 wV69i s-1(1)69 chromosome and ras m fw showed that the semi-lethal effect was close to the right of v and separable from it. An exact crossover unit value was impossible to obtain, due to the survival of an occasional mutant male. Males carrying the treated X chromosome were next mated with ras 1(1)L1 m f females. Lethal 1(1)L1 is located approximately 0.64 crossover units to the right of v, and s-1(1)69 lives in heterozygous combination with 1(1)L1. All recombinants involving the ras-v and v-m regions were tested for lethality. All males were tested for fertility as well. Of 24 male offspring, only one, carrying ras, proved to be a fertile, nonlethal recombinant. The occurrence of this ras male places s-1(1)69 to the left of 1(1)L1. The percentage of recombination between the two lethals was only 0.05%. Recombinational values within the v-m interval were not significantly altered by the presence of the lethal. A value of 2.9% (127/4314) was obtained for the
\text{v-m} \text{ interval as compared with the standard value of 3.1\%.}

When separated from \text{s-l(1)69}, \text{v}^{69i} \text{ acts as a normal v allele in both hemizygous and homozygous condition. The s-l(1)69 mutant produces an occasional viable male under optimal culture conditions, but is completely lethal as a homozygous female.}

In addition to the 92 phenotypically mutant F\text{1} males tested, another 258 F\text{1} males showing no phenotypically visible mutations were tested for female sterility and sex-linked recessive lethality. Of these 258 males, 108 were sterile. Although no female-sterile mutants were found, two semi-lethals were. Both of these were fractional mutants showing abnormal phenotypes, but were not covered by \text{v Y}^+\text{Y}. The mutants were small, with outstretched wings and a furrowed-like bristle effect. Dissection showed no motile sperm and testing showed all males to be sterile. None of the 3 major duplications used covered these semi-lethals.

A total of 6 more lethals were found, all of which were fractional but not covered by \text{v Y}^+\text{Y} and therefore were not in the region of interest.

\text{2000r-induced mutations: After treatment with 2000r and mating with C(1)DX females, 25 y}^2\text{cho}^2 \text{ irradiated males produced 1524 F\text{1} sons. Of these 1524 males, 5 were phenotypically mutant. These visible mutants included: (1) a white-eyed male, (2) a male with small, split-like}
eyes but showing no bristle effect, (3) a male mosaic for split-like roughness, having one "nicked" eye, (4) a Minute-like male and (5) a Delta-like male. All 5 of these mutants were sterile.

A total of 1401 F₁ males were tested for female-sterility and sex-linked lethality. Of the F₁ sons, 550 were sterile. Although no female-sterile mutants were found, 2 fractional lethals were recovered. One was not covered by the v Y y⁺ duplication. The other is covered by Dp v65b, Dp v⁺63i, and v Y y⁺. This places the lethal somewhere between 9F12 and 10A11. The lethal lives over 1(1)L1, 1(1)L8, and Df(1)v64f29, which includes salivary bands 9F3 through 10A2. This lethal must therefore be located somewhere between salivary bands 10A2 and 10A11. The lethal dies over Df(1)vL1 and Df(1)vL3. This narrows the location of the lethal to salivary band 10A2 through 10A4 or 10A5. When tested with 1(1)L12, it proved to be allelic. Thus the lethal must be located 0.49 crossover units to the right of v.

4000r-induced mutations: After treatment with 4000r and mating with C(1)DX females, 50 wild type males produced 2500 F₁ sons. None was phenotypically v. Of these 2500 F₁ males, 785 were tested for the presence of either sex-linked recessive lethal or female-sterile mutations. Of the 785 males tested, 324 proved to be sterile. Six lethals were found, all but 1 of which were fractional.
One fractional male-lethal mutant is covered by both \( Dp \, v^{+63i} \), and \( v \, y^+ \), but not by \( Dp \, v^{65b} \). This places the lethal somewhere between salivary chromosome bands 9F5 and 9F13, just to the left of the \( v \) locus.

A second fractional male-lethal mutant is covered by both \( Dp \, v^{65b} \), and \( v \, y^+ \), but not by \( Dp \, v^{+63i} \). This lethal must be located between salivary chromosome bands 10A10 and 10C2.

The other three fractional lethals were not covered by \( v \, y^+ \). However, one of these was covered by \( Dp \, v^{+63i} \), placing it between bands 9D4 and 9F5.

Two semi-lethals were also found. One was phenotypically normal, the other a visible mutant. This mutant shows variable expression, and appears to be dominant. Both females and males covered by the \( v \, y^+ \) show some abnormalities. The few uncovered males which survive are small with abnormal wing-vein effects similar to those seen in plexus and Delta mutants. \( Dp(1)v^{65b} \) does not cover the mutant, but \( Dp(1)v^{+63i} \) covers the mutant effect completely. The mutant probably is located somewhere between 9F5 and 9F13.

In the \( F_2 \) generation a mutant with a forked-like phenotype was found. This mutant proved to be allelic with the laboratory \( f \). It is male viable and salivary chromosome analysis showed it to be cytologically normal.

Altogether, following EMS and X-ray treatment, in
the examination of 5712 F$_1$ males and testing 2536 of them for the presence of male-lethal and female-sterile mutations, a total of 5 mutants were found to be covered by the vYy$^+$ chromosome. These include one male-viable v mutant, a simultaneously induced semi-lethal mutant located 0.6 map units to the right of v, one male-lethal mutant allelic with l(l)L12, a male-lethal mutant just to the right of v in salivary chromosome region 9F5 to 9F13, and a male-lethal mutant located between salivary chromosome bands 10Al1 and 10C2. No female-sterile mutants were identified.

Although Df v$^{65b}$ heterozygotes are sterile at 25°C, a few offspring survive at 18°C. Twelve Df v$^{65b}$ heterozygotes carrying Dp v$^{65b}$ produced 1792 offspring at 25°C, only 555 at 18°C. Twelve Df v$^{65b}$ heterozygotes carrying Dp v$^{+63i}$ produced 730 offspring at 25°C, only 225 at 18°C. Dp v$^{+63i}$ therefore restores fertility to nearly 50% of the value obtained for controls.
CHAPTER V

DISCUSSION

Detection of dominant female-sterile mutants: After treatment of males with either EMS or X rays, the typical procedure for recovery of the treated X chromosome is to mate the treated male with a balancer female which contains a crossover suppressor in the form of an inversion. The untreated X chromosome of the female will presumably cover any damage induced in the treated X chromosome of the male so that F1 females should be viable and fertile. Suppose, however, a dominant female-sterile mutation had been induced in the treated X chromosome. Such mutants would be lost during the above procedure.

The present experiment was designed specifically to recover such female-sterile mutants. After treatment with either EMS or X rays, males were mated with C(1)DX females carrying a special Y chromosome containing a duplication of salivary chromosome bands 9F5 through 10C1 or 10C2. This duplication includes the region of the X chromosome suspected of containing a dominant female-sterile locus. Theoretically, one should be able to recover both male-lethal and female-sterile mutants induced in this region.
At the same time, male-lethal and female-sterile mutants induced in chromosome regions not covered by the duplication should be eliminated.

No female-sterile mutants were found. This experiment was of such a magnitude that at least one or two should have been found. In the experiments of Lefevre (1967), 15 \( v \) mutants were found among 19,000 \( F_1 \) daughters produced following the irradiation of mature sperm with 4000r. These included 1 male-viable, 4 male-lethal, and 10 sterile \( v \) mutants. In other words, one out of every 1900 \( F_1 \) daughters carried a sterile \( v \) mutation. Included among 15,000 \( F_1 \) daughters produced by mature sperm irradiated with 2000r were 1 male-viable, 1 male-lethal, and 3 sterile \( v \) mutants. Here the frequency of sterile \( v \) mutants was one in 5000. Thus, at least 1 \( v \) sterile mutant could be expected among the 4024 \( F_1 \) male progeny of X-rayed males examined in the present experiment. It is possible, of course, that on the basis of chance alone no such mutants were recovered.

A semi-lethal mutant induced under the duplication:

A semi-lethal mutant, \( s-1(1)69 \), located approximately 0.6 map units to the right of \( v \) and covered by \( v Y y^+ \), was induced by EMS. This mutant is extremely inviable, and the few emerging males are sterile and phenotypically abnormal. Fahmy and Fahmy (1959) described two chemically induced mutants closely resembling \( s-1(1)69 \) phenotypically,
each located just to the right of \( v \) at 33.5. The first, dishevelled (dsh, 1-33.5), was described as follows: "Thoracic hairs deranged. One or more hairs abnormally curved. Wings are usually divergent, slightly upheld, and blistered. Eyes are ellipsoid, with some deranged ommatidia. Males are viable and fertile, females sterile." The second, thorny (tny, 1-33.5±0.3), was described as follows: "Very inviable, grossly deformed fly. Eyes markedly reduced in size, oval, very rough and dull red. Wings abnormal in shape, outspread and rarely fully expanded. Males sterile." Either of these mutants could possibly be allelic to \( s-1(1)69 \), but tests for allelism could not be undertaken since the Fahmy stocks are not available.

Both \( s-1(1)69 \) and the Fahmy mutants affect male viability, fertility, and sexual development. All show varying degrees of phenotypic expression, and all might very well be associated with a sex-determining locus rather than a female-sterility locus. The fact that no female-sterile mutants covered by the duplication were found may signify that a sex-determining locus, when mutant, produces male as well as female sterility, and that the duplicated region of the \( vYy^+ \) chromosome is unable to cover the mutant effect. This type of mutant will be impossible to isolate if the locus is really involved with sex determination, not simply female
sterility.

Only under optimum culture conditions does $s-1(1)69$ produce a few viable, although abnormal and sterile, males. No females homozygous for $s-1(1)69$ have yet been found. This mutant, thus, acts as a semi-lethal recessive visible in the male, but as a recessive lethal in the female. Perhaps $s-1(1)69$ is a recessive mutant of the hypothetical female-sterile or sex-determining locus. At other loci, both dominant and recessive alleles are known. One example is Stubble, a dominant mutant and its recessive allele, stubbloid; a second example is Star and its recessive allele, asteroid, located 0.02 crossover units to its right (Lewis 1945).

In addition to $s-1(1)69$, 4 lethals, $1(1)L12$, $1(1)L8$, $1(1)Q66$, and $1(1)L1$, have been located in a short interval between 0.5 and 0.6 crossover units to the right of $v$ (Lefevre 1969). Both $1(1)L1$ and $s-1(1)69$ are located in the region between salivary chromosome bands 10A7 and 10A11. These 5 mutants appear to be completely independent. They are not allelic and are separable by crossing over.

Complex loci: Welshons (1965) studied the complex Notch ($N, 1-3.0$) locus. A few comparisons can be drawn between this locus and a hypothetical sex-determining locus involving $s-1(1)69$. Notch mutants are deficient for band 3C7, show a dominant phenotype in the female,
and act as recessive lethals. A series of completely recessive, non-Notch lethal alleles also occur in the Notch locus. Six recessive visible mutant alleles are also known at the _N_ locus. Three are eye mutants, including split (spl) and facet (fa), and 3 are wing mutants. The wing mutants are noncomplementary and show an intermediate mutant expression. The 3 eye mutants are also noncomplementary except for _spl_ and _fa_. All wing mutants, however, are complementary with eye mutants. Why then are these two apparently independent groups of mutants considered to be alleles of _N_?

Facet-notchoid, _fa^no_, a male-viable allele of _fa_ with notched wings, is lethal when heterozygous with _N_. The other 5 recessive visible mutants show various phenotypic interactions with _N_. This is the basis for their being grouped within one gene. Is it possible that so many different alleles, each expressing a distinctly different phenotype, can all be included in one functional gene? Welshons stated, "Visible Notch-like males could result from partial rather than complete inactivation of the gene." _N/fa_ heterozygotes show a facet phenotype which is exaggerated in comparison to homozygotes. Welshons stated, "... _fa_ causes a reduction in genetic activity, hence, as far as total activity is concerned _N/fa_ produces less product than _fa/fa_ and is accordingly more mutant in phenotype." Mutant _s-1(1)69_ could be a
mutant like \( fa \), related to the hypothetical sex-determining mutant in the same way that \( fa \) is related to \( N \). If this mutant is an allele of the hypothetical female-sterile or sex-determining locus, deficiency for which is dominant in its effect, then an analysis of its relationship to its mutant alleles would be virtually impossible.

Alleles within the Notch locus, like those within the hypothetical female-sterile locus, show different phenotypes. Welshons described the Notch locus in terms of a cistron producing one protein or polypeptide. Although the intracistronic visible mutants affect the same protein, they could have different phenotypes if the protein is involved in more than one reaction. More than one change can occur in a given protein structure, affecting its relationship to different systems in different ways.

**Mosaic mutants:** In the examination of approximately 6000 \( F_1 \) males and testing over 2500 of them, only 5 mutants were induced under the duplication. Three of these were fractional mutants. A total of 117 mosaic mutants not covered by the duplication were induced. Most of these were sterile or bred phenotypically wild type. Eleven lethals and 4 semi-lethals which were fractional and not covered by the duplication were also induced. This experiment was designed to recover male-lethal and female-sterile mutants covered by the \( vYy^+ \) duplication.
The above results indicate this procedure was not an efficient way to recover a number of mutations in this specific chromosome region.

Studies made by Lee, et al. (1967) help explain why many mutants induced outside the covered region survived. Lee stated that there is no correlation between the proportion of mutant cells in somatic tissue and in the germ line. Mosaic germ lines represent cases where the sample of cleavage nuclei destined to become the germ line came from the border between mutant and nonmutant nuclei. In such cases $F_1$ phenotypic mutants may breed wild type while germ line mutants may not be identified until the $F_2$ generation. The procedure used in the present experiment insured only that $F_1$ males carrying a recessive lethal or dominant female-sterile mutant would live. $F_1$ males carrying in their germ line a lethal induced anywhere along the $X$ chromosome will also survive.

**Summary:** No female-sterile mutants were recovered. This may merely mean an insufficient number of chromosomes were tested. The above procedure is a time-consuming one, however, since three generations must be raised before such a dominant female-sterile mutant can be identified. If this locus is in fact a sex-determining locus also affecting male viability and fertility, such a mutant is not recoverable. This possibility is implied by the fact that all 3 of the visible mutants known to be present
in this region, including s-1(1)69 and Fahmy's mutants, have an effect on viability and fertility in both sexes. A more successful method for isolating such a mutant might be to test only F1 v mutant males. It is known from the work of Lefevre (1967, 1969) that the hypothetical female-sterile mutant is closely associated with the v locus, at least cytologically near to it. The one interesting mutant found in the present experiment, s-1(1)69, was accompanied by a simultaneously induced v mutant. Whether or not s-1(1)69 is related to the hypothetical dominant female-sterile mutant cannot be determined until the dominant female-sterile mutant is recovered.
BIBLIOGRAPHY


