CHEMICALLY-INDUCED MALE RECOMBINATION AND CHROMOSOMAL ABERRATIONS IN *Drosophila melanogaster*

By

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(L-90-B5-163-M)

Thesis
SUBMITTED TO THE PUNJAB AGRICULTURAL UNIVERSITY IN
PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE IN GENETICS

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My Revered

Mumy & Dady

&

My Loving

Doll & Dicken

CERTIFICATE I

This is to certify that this thesis entitled **" Chemically-induced male recombination and Chromosomal aberrations in *Drosophila melanogaster*"** submitted for the degree of **Master of Science** in the subject of **Genetics** (Minor: Plant Breeding) of the Punjab Agricultural University, Ludhiana is a bonefide research work carried out by **Gursharan Kaur** (L-90-BS-163-M), under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help recieved during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that the thesis entitled, " **Chemically-induced male recombination and chromosomal** aberrations in *Drosophila melanogaster* " submitted by Gursharan Kaur (L-90-BS-163-M) to the Punjab Agricultural University, in partial fulfulment of the requirment for the degree of **Master of Science** in the subject of **Genetics** (Minor: Plant Breeding) has been approved by the students Advisory Committe after an oral examination on the same, in collaboration with an External Examiner.

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ACKNOWLEDGEMENTS

I feel great pleasure to express my sincere gratitude to Dr. G.S. Miglani, Ph.D (Howard), Associate Professor of Genetics, Punjab Agricultural University, Ludhiana, for his devotion, constructive criticisim, constant encouragement and invaluable help in every phase of planning, experimentation and preparation of this manuscript.

My sincere thanks are also due to Dr. Ashok Kumar, Geneticisit, Dr. G.S. Mahal, Wheat Breeder and Dr. Gurdev Singh, Senior Oil Seed Breeder for their keen interest in the progress of my work and for critically reviewing this thesis. I am obliged to Dr. J.L. Minocha, Senior Geneticist, for his advice and useful suggestions at various stages of this work.

I am indebted to my dear parents for their infinite love and trust they bestowed upon me. Without their support, I would have been no where. I will always cherish the love and affection given to me by my loving DOLL and DICKEN. I also convey my regards to my Pawar uncle and dear Karnail Mama ji for their love and inspiration in my life.

I am grateful to Mrs. Harjit Miglani, Jemmy and Simmy for their love and affection.

It gives me pleasure to convey my affectionate feelings to my dearest friend Nimy for her warm affection and confidence.

I am grateful to my special freinds of batch '90' Hardeep, Nirlep, Ranjit, Nalini, Bandana and Charanjit for the wounderful time I had in their company. Thanks are also due to Navjot, Mini and Poonam for their nice companionship and everwilling help.

My special thanks to Shri Gurmail Singh and Shri Malkiat Singh for their day to day help in the labortary.

I also wish to thanks Dr.R.C. Woodruff for sending us the *al dp b cn* stock of *Drosophila melanogaster* from Mid-America Drosophila Stock Centre, Bowling Green State University, Bowling Green, USA. A word of "sorrow" for *Drosophila* flies who gave their lives for me.

Facilities provided by the Punjab Agricultural University during the course of this investigation are also duly acknowledged.

January 27, 1993

Gursharan Kaur

(GURSHARAN KAUR)

Title of the thesis : Chemically-induced male recombination and chromosomal aberrations in *Drosophila melanogaster*
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Name of the Major : Dr. G.S. Miglani
Advisor and designation : Associate professor of Genetics
Major Subject : Genetics
Minor Subject : Plant Breading
Degree to be awarded : M.Sc.
Total pages in the thesis : 84 + vii
Name of the University : Punjab Agricultural Univeristy Ludhiana

ABSTRACT

Maleic hydrazide (MH), a growth regulator and potent chromosome breaking agent, was used to test its ability to induce recombination in males of *Drosophila melanogaster* and to explore role of chromosomal aberrations with male recombination. For this, FS$_1$ (+/*aristaless dumpy black cinnabar, al dp b cn*) larvae were fed on 0.5% MH-mixed food in ratio 1:9 during the entire larval life. In pooled TC$_1$ progeny of 11843 flies, 211 (84 male + 127 female) recombinants were recovered. All the TC$_1$ recombinant males were genotypically/ phenotypically verified. Recombinant type *b cn* was the most and *al dp* the next most predominant recombinant phenotype. Male recombination was non-reciprocal in *dp-b* (1.661%) and *b-cn* (0.11%) regions whereas reciprocal recombination was observed in *al-dp* (0.372%) region. Fifty homozygous male recombinant lines (HMRLs) were constructed from MH-induced TC$_1$ recombinant males. The salivary chromosomes of progeny larvae of 44 of these HMRLs were examined which revealed no chromosoml aberration. Out of the 25 HMRLs studied in reciprocal crosses for their potential to produce male recombination, 82.0% of the crosses exhibited this potential. In this study, a total of 15 chromosomal aberrations were detected in F$_1$, TC$_1$, TC$_2$, FS$_1$ and FS$_2$ larvae; 10 of these were included in chromosome 2L out ot which 8 overlapped *dp-b*, the region in which highest frequency of male recombination was recorded. The present observations thus suggest a direct or indirect role of chromosomal aberrations in male recombination in *D. melanogaster*.

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CONTENTS

CHAPTER I

INTRODUCTION

Recombination is an important evolutionary force. It is one of the important consequences of meiosis and is responsible for release of genetic variability in natural populations. Genetic recombination is a fundamental property of all living systems starting from the RNA-containing viruses and ending with the higher plants and animals. Recombination involves the physical exchange of material between chromatids of homologous chromosomes. Non-parental combination of alleles, which are obtained in segregating generation are called "recombinants". In sexually reproducing animals and plants the recombination of linked genes in brought about by crossing-over. Any effort towards understanding the process of recombination could be of value for increasing genetic variability in cultivars and domestic animals.

Frequency of recombination is not equal in both the sexes of all the organisms. For example, crossing-over is known to be absent in males of *D. melanogaster* (Morgan, 1912, 1914). One of the approaches to understand the process of genetic recombination is to induce it in a system, such as males of *D. melanogaster,* where it is normally absent. *Drosophila* is an excellent test system for studies on recombination also because it is easy to handle, has a shorter life cycle and produces a large progeny. Also because of existence of large number of genetic markers and availability of the requisite stocks of *D. melanogaster*, this species provides an ideal material for understanding the process of recombination.

Hiraizumi (1971) found a low but significant level of spontaneous male recombination in *D. melanogaster* for natural populations. The mechanism underlying the induction of male recombination is still not well understood. By now a large number of physical agents, namely, γ-rays, neutrons, physical stress, radiofrequency, temperature, ultraviolet light and X-rays and chemicals agents, namely, acridine orange (AO), bleomycin (BLM), chloroquine phosphate (CHQ), dihydroxy dimethyl peroxide (DHMP), ethidium bromide (EB), 8-ethoxy caffeine (EC), ethyl methanesulphonate (EMS), formaldehyde (FA), hydroxylammonium sulphate (HAS), methyl methanesulphonate (MMS) mustard gas (MG), nitrosoethyl urea (NEU) and nitrosomethyl urea (NMU) are known to induce recombination in males of *D. melanogaster* as reviewed by Thapar (1982), Mohindra (1984), Ferres *et al.* (1984), Singh (1989), Preet (1989), Singh (1992), Miglani (1993).

Recombination products resulting from a crossing-over event are expected to appear in a test cross progeny in 1:1 ratio. This is termed as "reciprocal" recombination. If the recombinant products do not appear in 1:1 ratio, recombination is termed as "non-reciprocal". Non-reciprocal recombination is an uncommon phenomenon. Most of the spontaneous and induced male recombination events have been observed to be non-reciprocal in nature.

In addition to various other chemicals reported in literature, EMS (Miglani and Thapar), 1983, Miglani and Mohindra, 1986a,b and Singh, 1992), CHQ (Miglani and Thapar, 1983a) and HAS (Miglani *et al*, 1990) and FA (Miglani and Preet, 1990) have been observed in our laboratory to induce non-reciprocal recombination in males of *D. melanogaster.* Male recombination is known to be associated with sterility, Seqregation distortion and sex-ratio imbalance which includes most of symptoms of a syndrome known as "hybrid dysgenesis" in natural populations of *D. melanogaster.*

Recent studies suggessted that male recombination and other phenomenon associated with it are different aspects of the same phenomenon, i.e., chromosome breakage (Miglani and Mohindera, 1986b). Chromosomal aberrations were detected in the progenies of EMS (Miglani and Mohindra, 1985) and HAS (Singh,1989) induced TC_1 male recombinants of *D. melanogaster.*

When male of homozygous male recombinant lines (HMRLs) derived from HAS-induced male recombinants were crossed to females from an aberration-free stock, the chromosomal aberration were not recovered in recombinant heterozygotes (Miglani *et al.*, 1990). Miglani and Singh (1992a) also did not observe any chromosomal rearrangement in the progeny larvae of the HMRLs derived from EMS-induced TC_1

male recombinants. Involvement of chromosome breaks/ aberrations in male recombination in *D. melanogaster* is still an open question.

Maleic hydrazide (MH), a growth regulator, is also a potent chromosomse breaking agent. Parkash and Miglani (1978) observed chromosomal aberrations with MH in salivary chromosomes of *D. melanogaster*. With MH no chromosomal aberration was observed in chromosome 2L. It was thought worthwhile to simultaneously study the induced chromosomal breaks/aberrations and male recombination in *D. melanogaster* to find possible relationship between these phenomenon using MH as a probe.

Thus, the present investigation was carried out with the following objectives:

(1) To induce male recombination and chromosomal aberrations with maleic hydrazide in *D. melanogaster* and confirm genotypes/phenotypes of the male recombinants induced.

(2) To examine the salivary chromosomes of the larvae at different stages of the experiment and finally of the progeny larvae of the HMRLs for the presence of chromosomal aberrtions.

(3) To find out if any of chromosomal aberrations descends from an F_1 male to the generation when HMRL was obtained.

CHAPTER II

REVIEW OF LITERATURE

2.1 Genetic Recombination

Gentic Recombination is one of the most important consequences of meiosis and is instrumental in generating genatic variability. Without Genitic recombination, the content of each individual chromosome would be irretrievably fixed in its particular alleles, changeable only by mutation. By shuffling the genes recombination allows favourable and unfavourable mutations to be separated and tested as individual units in new assortments. From the long perspective of evolution, a chromosome is a bird of passage which has a temporary association of particular alleles. Recombination is responsible for for this flighty behaviour.

Genetic Recombination is defined as the redistribution of information inherited from the parents in the progeny. Recombination involves the physical exchange of materials between chromatids of homologous chromosomes. Non-parental combination of allels which are obtained in F_2 generation are called recombinants. In sexually reproducing animals and plants the recombination of linked genes is brought about by crossing-over whereas recombination of the unlinked genes is based on the mechanism of free combination of chromsomes in meiosis.

2.2 Theories of Genetic Recombination

Theories explaining mechanism of recombination may be divided in to three general groups: breakge and copying, complete copy choice and breakage and reunion.

2.2.1 Breakgae and Copying

This theory given by Bellings (1933) purposed that the recombinant chromosome is formed by utilizing the physical section of parental chromosome and by copying the other. Thus the recombinant chromosome due to the breakage, at the same time contains a part of the old and new chromosomes synthesized by copying the other strand.

2.2.2 Complete copy choice

A modified version of Belling's theory was given by Leaderberg (1955). The entire chromosome arises from newly synthesized secton which has copied a part of their sequence from the section of other parental chromosome. This theory signifies that the repication is at the chromatid level.

2.2.3 Breakage and reunion

According to this theory purposed by Darlington (1937), the two places of parental chromosomal information that are combined in the recombinant chromosome arise from the physical breaks in the parental chromosomes with subsequent physical exchange. The paired parental chromosomes break and rejoin at meiosis resulting in the formation of "chiasmata". The Chromosome exhcange materializes at these chiasma. In this case recombination of new DNA molecules since the information entering the recombinants is merely transfered from the parentals.

2.3 Differential Recombination in Females and Males of the Species.

Frequency of recombination is not same in both the sexes of all the species. For example, crossing-over is known to be absent in males of *Drosophila melangaster* (Morgan, 1912, 1914) and females of *Bombyx mori* (Darlington, 1937). Recombination in males of *Drosophila* was considered to be absent untill HIraizumi (1971) found a low but significant level of spontaneous male recombination in *Drosophila melanogaster* from a natural population. Following criteria can be used to disinguish between female and male recombination in *D. melanogaster* (Miglani, 1993).

1. Both non-reciprocal as well as reciprocal recombination are known in females and males; non-reciprocal male recombination events being predominant over reciprocal male recombination events. (Miglani and Thapar, 1983b)
2. All the recombinants resulting from crossing-over in females of *D. melanogaster* are confirmed whereas all of those resulting from recombination in males of the same species are not confirmed genotypically/phenetypically (Miglani and Mohindra, 1986a).

3. Female recombination is generally not associated with any aberrant trait whereas the male recombination is known to be associated with sterility, chromosome breakage, sex-ratio daviation and segregation distortion (Hiraizumi, 1971, 1977; Miglani and Thapar, 1983a; Miglani and Mohindra, 1986b).
4. Shull and whitinghill (1934), while investigating the effect of heat on male recombination in *D.melanogaster*, observed that induced crossing-over in males, unlike females, was not distributed throughout the length of chromosome at random but was localized only to some particulars regions. This was termed as "clustering".

2.4 Spontaneous Male Recombination in *Drosophila*

Spontaneous male recombination events have been observed to be of both reciprocal as well as non-reciprocal type. Cross overs arising from non-reciprocal events are clustered while those arising from reciprocal events are not clustered. Spontaneous male recombination is known to be associated with sterility (Miglani and Thapar, 1983a) sex-ratio imbalance and segregation distortion (Miglani and Mohindra, 1986b).

Spontaneous male recombination in natural populations of *D. melanogaster* may be caused by male recombination factors (MRFs) that are located on chromosome 2 (Yannopoulous *et al.*, 1981,1982). Some inversion systems do

(Yannopoulous, 1978b) where as some others do not (Mahajani, 1971) affect spontaneous male recombination in natural populations of *D. melanogaster*. In dysgenic hybrids male recombination was related with females sterility, found to occur in clusters and pre-meiotic in origin (Woodruff and Thompson, 1977). Singh and Singh (1990) has described several cases of spontaneous male recombination in different species of *Drosophila*, including a genotypically unique species *D. ananassae*.

2.5 Induced Male Recombination in *Drosophila*

Incidents of both reciprocal and non-reciprocal recombination in *Drosophila melanogaster* males have been reproted by use of physical and chemical agents.

2.5.1 Chemicals agents

Reciprocal as well as non-reciprocal male recombination was reported in *D. melanogaster* with various chemicals agents, namely, acridine orange (AO), bleomycin (BM), chloroquine phosphate (CHQ), dihydroxy dimethyl peroxide (DHDP), ethidium bromide (EB), ethyl methanesulphonate (EMS), formaldehyde (FA), hydroxylammonium sulphate (HAS), maleic hydrazide (MH), methyl methanesulphonate (MMS), mustard gas (MG), nitroosoethyl urea (NEU) and nitrosomethyl Urea (NMU). Higher cross over frequency was observed when a chemical was administered in food rather than by injection (Zvarik and Bohmona, 1978). Miglani (1993) has reviewed the results obtained by different workers using these chemicals agents, Non-reciprocal male recombination was accompanied by other phenomenon like segregation destortion and sex-ratio deviation and these phenomenon were transmited to next generation; these were considered different aspects of the same phenomenon, i.e., chromosome brekage (Miglani and Mohindra, 1986b).

Instances of non-reciprocal male recombination were aound to be more frequent than those of reciprocal male recombination, Most of the reports reveal second chromosome region *dp-b* of *D. melanogaster* to be most sensistive to the induction of male recombination with different chemicals. No relationship was found between EMS-induced chromosome breakage and male recombination. Most of the male recombination events were pre-miotic in origin (Miglani *et al.*, 1990). Chromosomal aberration were detected in progeny larvae of EMS (Miglani and Mohindra, 1985)- and HAS (Miglani *et al.*, 1990)- induced TC_1 recombinant males. No aberration was detected in the homozygous male recombinant lines (HMRLs) constructed from chemically induced recombinant males (Miglani *et al.*, 1990). Some of the HMRLs exhibited inherent potential of inducing male recombination.

2.5.2 Physical agents

Among the physical agents reciprocal and non-reciprocal male recombination was observed in *D. melonagaster* with γ-rays, neutrons, physical stress, radiofrequency, temprature, ultra violet light and X-rays using several second and third chromosome genetic markers. Miglani (1993) has reviwed the recombinogenic effects of these physical agents.

Non-reciprocal events were reported to be more frequent and accompanied by sex-ratio distortion and sex-ratio deviation. Majority of cross overs originated from pre-meiotic events whereas a small fraction results from meiotic male recombination depending upon the stage at which the treatment was given.

2.6 Factors Associated with Male Recombination

Spontaneous as well as induced male recombination has been associated with various aberrant traits, such as male and female sterility, sex-ratio deviation, segregation distortion and hybrid dysgensis, in *D. melanogaster*.

2.6.1 Sterility

Miglani and Thapar (1983a) suggested an inverse association of male sterility with EMS- and CHQ- induced male recombination in *D. melanogaster.* Miglani and Mohindra (1986b) observed a considierable degree of sterility induced by EMS. It was suggested that induction of greater degree of complete sterility, partial sterility and dcrease in longevity of adults subsequent to treatment with EMS may be attributed to prologed residual effect of this chemical (Miglani and Thapar, 1983a)

2.6.2 Segregation distortion

According to Peacock and Ericksy (1965) only half of the sperms of *Drosophila* males were capable of fertilizing female eggs and incase of segregation distortion, this half included mainly those gametes carring the wild type chromosome. According to Kidwell and Kidwell (1975b), segregation distortion seemed to be associated with male recombination. Woodruff and Layman (1980), reported that in natural populations male recombinations and miotic derive were due to elements on the second chromosome and the region between *b* and *cn* contained segregation distortion system. Miglani and Mohindra (1986) reported association of non-reciprocal male recombination with segregation distortion.

2.6.3 Sex-Ratio deviation

Miglani and Mohindra (1986b) observed that sex-ratio deviation for *dp b cn* and *b cn* phenotypes, induced in F_1 males was transmitted to next generation when caused by EMS but not when these abnormalities were induced by "physical stress" Miglani and Mohindra (1987) explained "physical stress" as followss : After 54h of egg deposition, the developing *Drosophila* larvae are flushed out with water and then physically transferred with a camel hair brush on to the standared food medium. After allowing the larvae to feed there for 32h, they are again flushed out and transferred on to the standard food medium . Each larva thus undergoes two physical transfers during its life time which is reffered to as "physical stress".

2.6.4. Hybrid dysgenesis

When *D. melanogaster* females from strain maintained in the labortary for several generations are mated with males from natural population, it is frequently observed that their F_1 progenies show one or more aberrant traits, such as sterility, mutation, chromosome breakage and male recombination. This syndrome of aberrant traits is called "hybrid dysgenesis" (Sved, 1976 ; Kidwell, 1979).

Two systems responsible for "hybrid dysgenesis " have been described in *D. melanogaster*. They are (1) The P-M system and (2) The I-R system. The P-M system is recognised by induction of sterility and male recombination in the progeny of crosses between M-Strain females and P-Strain males (Kidwell *et al.*, 1977). Some associated dysgenic traits include mutation, transnmission-ratio distortion, non-disjunction, chromosomal aberrations and increased frequeneies *of female recombination. The I-R system of hybrid dysgenesis* was first discovered by Picard (1971). This system has been indentified on the basis of specific type of sterility called specific female sterility. The role of transposable (mobile) genetic elements in hybrid dysgenesis has been indentified.

2.7 Pre-meiotic and Meiotic Male Recombination in *D. melanogaster*.

Chiasma formation is associated at both male and female meiosis with the formation of structures termed as "synaptonemal complexes" during zygotene and pachytene stages. Meyer (1952) made it clear that the synaptonemal complex was seen only in females but not in males. Ray-Chandhri and Kale (1965, 1966) studied spontaneous recombination in the males of *D. ananassae* by employing second chromosome makers (*cu, b, se*) and reported it to be meiotic in origin.

Hirazumi *et al.* (1973) suggested that considerable fraction of female recombination is pre-meiotic in origin. Yaunopolous and Palecanos (1977) observed similar results and favoured the assumption that male recombination in *D. melanogaster* is pre-meiotic in origin.

2.8 Mechanism of Induction of Male Recombination in *D. melanogaster*

The cross overs obtained in the *dp-b* regin of the left arm of chromosome 2 were attributed to isolocus breaks in homdogous chromosomes followed by exchanges (Sharma and Swaminathan, 1968).

Male recombination in *D. melanogaster* could occur, as suggested by Woodruff and Thompson (1977), by classical crossing-over as in females, or by chromosome breakage and reunion events. Since Miglani and Thapar (1983a) while studying male recombination in *D. melanogaster* failed to recover complementary recombinants with EMS and CHQ in equal frequency, they suggested that male recombination events probably occured not by classical crossing-over but by preferential breakage and reunion of chromosome.

Miglani and Mohindra (1986a) also failed to recover reciprocal recombinants in *dp-b* region of *D. melanogaster* in equal frequency with EMS. Some of the chromosomal aberrations detected in pregeny larvae of EMS (Miglani and Mohindra, 1985) and HAS (Singh, 1989) induced male recombinants overlapped the *dp-b* region. This strongly suggested the involvement of chromosome breakage and reunion in male recombination in *D. melanogaster*.

2.9 Chromosomal Aberrations and Male Recombination in *Drosophila*.

According to Voelkar (1973), male crossing-over and the occurence of unique chromosomal aberrations, mostly inversions, in *D. melanogaster* may be under same genetic control. Engels (1979) observed that in an attached X-chromosome line, level of male recombination in M-Strain was not homogeneous, i.e., recombinants were clustered and some large clusters included only one recombinant type. Lutken and Baker (1979) did not observe significant increase in male recombination over the control by deficiency.

Chromosomal aberrations may or may not be have an effect on induction of recombination in males of *D. melanogaster*. *Curly* inversions did not influence X-ray-induced spermatogonial crossing-over (Whittinghill, 1947). Non-reciprocal recombinantion was observed in *Freckled (Frd)* adults in the presence of an inversion homologous chromosomes (*Cy* and *Pm*) (Berrigozi, 1965).

Yaunopoulous and Zachropoulou (1980) reported that a second chromosome male recombinant factor, 31.1MRF induced male recombination in both the 2nd and 3rd chromosomes. Miglani and Mohindra (1985) found that there was no chromosomal abnormality in the salivary chromosomes of pregeny larvae of TC_1 recombinant males from the control experiment but inversions and deficiencies were found in the *dp-b* and *b-cn* regions of chromosome 2, in the EMS experiment.

Miglani and Singh (1992a) did not observe any chromosomal rearrangement in the pregeny larvae of HMRLs derived from EMS-induced TC_1 recombinant males.

2.10 Male Recombination in Homozygous Male Recombinant Lines of *Drosophila*.

Spontaneous male recombination and visible mutations were observed in *st-cu* and *st-*P^D regions of second chromosome derived from an isogenic line, ID (Kidwell and Kidwell, 1975b). Woodruff and Thompson (1977) observed that all the recombinant males bred true as second chromosome recombinants in the recombinant lines. They also observed equal recovery of reciprocal exchanges in the *b-ch* and *cn-bw* regions but not in the *dp-b* region.

Miglani and Singh (1992b) constructed homozygous male recombinant lines (HMRLs) from 34 EMS-induced TC1 recombinant males of *D. melanogaster*. Male/female from an HMRL was crossed to a female/male from complementary stock and the F_1 males thus obtained were crossed with *al dp b cn* females. The ability of HMRLs, constructed form chemically-induced male recombinants to produce male recombination was studied for the first time by these workers. Out of 68 test cross progenies studied in this way, 13 (19%) revealed male recombination, frequency ranging from 3.10 to 18.07 per cent. The results showed that some of the HMRLs derived from EMS-induced TC_1 male recombinants possessed inherent ability to produce male recombination (Miglani and Singh, 1992).

2.11 Action of Maleic hydrazide

Maleic hydrazide is widely used as a herbicide, fungicide and growth regulator. It is one of the verious reagents which induces breaks in the chromosomes of some plants at specific region recognizable as heterochromatin (Nasrat, 1965). It also inhibits growth in several species of plants (Evans and Scott, 1964).

Maleic hydrazide was shown to have mutagenic effect on plants, *Allium Cepa*, *Vicia faba*, *Nicotiana tabaccum*, *Hordium vulgare*, etc. According to Cortes *et al.* (1987), the cytogenetic action of maleic hydrazide in many respects resembles that of bifunctional alkylating agents.

Nasrat (1965) showed that maleic hydrazide was chemical mutagen in *Drosophila melanogaster*. According to him, most advanced atage in larval testis responded to maleic hydrazide. Nawar and Nagaty (1979) determined that treatment of females of *D. melanogaster* with caffeine or MH resulted in slight increase in either dominant lethals or sex-linked recessive lethals.

Parkash and Miglani (1978) for the first time reported the cytological effects of maleic hydrazide in *D. melanogaster*. These workers used 0.5% dose of maleic hydrazide which was administered through larval feeding to study its effects on salivary chromosomes of *Drosophila*.

They observed that MH has mainly yeilded heterozygous paracentric inversions. Induction of such inversions, therefore, has been taken as a measure of chromosomal damage. Every affected larva carried on the average 1.66 inversions with MH. Total 75 larvae were dissected and out of 4496 cells examined 10 inversions of eight types were observed. Only one inversion was centarlly located on X chromosome. The MH-induced inversions were located on different arms in such a way that no segment seemed to have been affected in particular. Two typesa of inversions induced with MH were similar to these observed with MMS.

Breakage-union pointer of inversions with MH when mapped on salivary chromosome map of Bridges (1936) indicated that loci 50A, 51B, 52E, 53F and 54D of the second chromosome and 64F, 66B, 67C, 69F, 88C, 91C, 92C, 94C, 95E and 96E of the third chromosome of *D. melanogaster* showed high frequency of breaks.

Ability of maleic hydrazide as an inducer of recombination in males of *D. melanogaster* is still unknown and hence is the subject of the present investigation.

CHAPTER III

MATERIAL AND METHODS

3.1 Materials

Two stocks of *Drosophila melanogaster* were used in the present investigation: a standard laboratory stock, oregon - K, and a mutant stock homozygous for three second chromosome recessive markers, *aristaless* (*al*:2-0.4), *dumpy* (*dp*: 2-13.0), *black* (*b*: 2-48.5) and *cinnabar* (*cn*: 2-57.5). Gene Symbols and map distances are as given by Lindsley and Zimm (1985).

Maleic Hydrazide (MH), with chemical formula $C_4H_4N_2O_2$, molecular weight 112.09, manufactured by Kemphasol, Bombay (batch no. PB 2277), was used as probe.

3.2 Preparation of Probe Solution

Parkash and Miglani (1978) induced chromosomal aberrations in *Drosophila melanogaster* using 0.5% MH. It was, therefore, decided to use the same dose of MH in the present experiments, at 25°C.

To prepare 0.5% solution of the probe, 500 mg of maleic hydrazide was dissolved in phosphate buffer (pH 7.0) to have final volume of 100 ml. The phosphate buffer sloution of pH 7.0 was prepared by mixing 39ml of solution A and 61ml of solution B. Solution A was prepared by mixing 27.8g of monobasic sodium phosphate ($NaH_2 PO_4.2H_2O$; Mol.Wt. 156.01) per litre and solution B was prepared by mixing 53.65 g of dibasic sodium phosphate (Na_2HPO_4; Mol. Wt. 142.0) per litre. The resultant solution was diluted to 200 ml and its pH confirmed on Systronics pH meter, type 331.

3.3 Food Medium

Standard *Drosophila* food medium consisted of following ingredients.

Agar agar	24 g
Yeast	24 g
Sugar (Desi)	64 g
Maize powder	72 g
Water	1500 ml
Propionic acid	2 ml

3.4 Treatment Procedure

Since *Drosophila* larvae are voracious eaters, treatment was given by rearing them on food medium mixed with MH in ratio 9:1. Two ml of 0.5% MH solution was added to 18 g of standard food medium, and was put in a glass vial of 3" x 1" size.

Forty-five to fifty homozygous *al dp b cn* females were crossed with approximately the same number of wild type (Oregon-K) males in the vial containg 0.5% MH mixed medium. Control experiments, where no MH was mixed to the food medium, were also done simultaneously. All the experiments were conductd at 25°C. A number of untreated and MH-treated third instar F_1 larvae were randomly selected for examination of salivary chromosomes. The adult males from F_1 pregenies were isolated and used for making further crosses.

3.5 Crosses of F_1 Males

A two-day old F_1 male was crossed with three *al dp b ch* virgin females on normal food to get the first test cross progeny (TC_1). This cross was replicated 2 to 3 times to get as large a TC_1 prageny as possible. From the progeny of every F_1 male, eight to ten TC_1 larvae were randomly selected for examination of salivary chromosomes. The adult male and female flies of different phenotypes appearing in TC_1 pregeny of each untreated and MH-treated F_1 male were recorded separately. The TC_1 male recombinants were saved for further experimentation.

3.6 Crosses of TC_1 Recombinant Males

Each TC_1 recombinant male was crossed with 3 to 4 *al dp b cn* females to obtain second test cross generation (TC_2) for confirming their genotypes/phenotypes. A TC_1 recombinant male was considered as confirmed if it produced recombinant and *al dp b cn* type flies in its TC_2 progeny. Six to eight TC_2 larvae from the progeny of every TC_1 recombinant male were randomly selected for the study of salivary gland chromosomes. The male and female flies of different phenotypes appearing in TC_2 progenies were recorded.

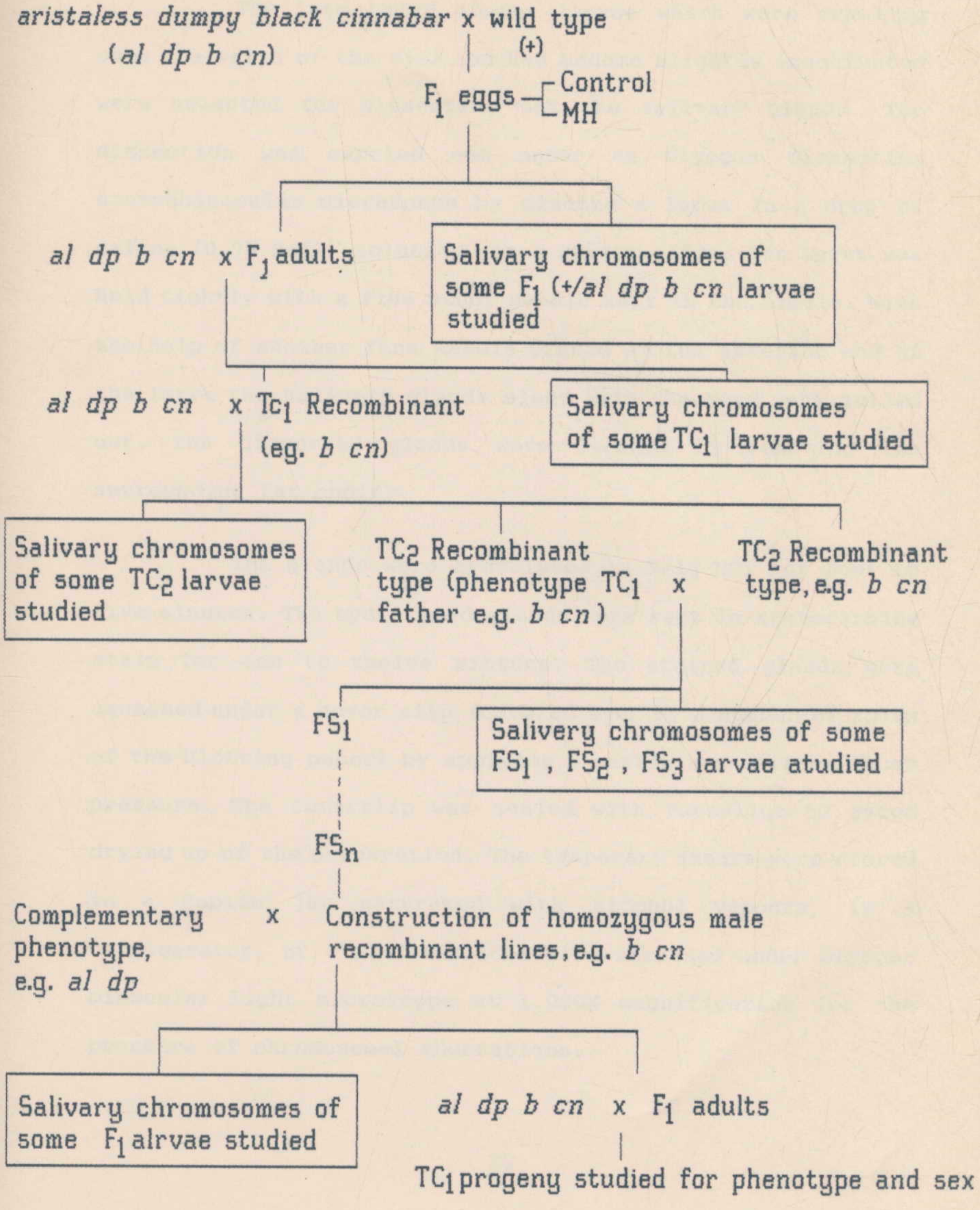
3.7 Construction and Study of Homozygous Male Recombinant Lines

The TC_2 adult male and female flies produced by a particular recombinant male and having phenotype of their recombinant TC_1 father were pair-mated to obtain first full sib generation (FS_1). Salivary chromosomes of five or six FS_1 larvae were examined from each progeny. Appropriate pair-matings were conducted and FS_2, Fs_3, etc. obtained till separate lines homozygous for all the TC_1 recombinant males were obtained. Henceforth these lines wille be referred to as homozygous male recombinant lines (HMRLs).

Five to six males from a particular homozygous recombinant line were crossed with the same number of females from an aberration-free complementary laboratory stock and vice-versa. Eight to ten F_1 larvae emerging from all these crosses were dissected out for the study of salivary chromosomes. The F_1 adults were screened for phenotype and sex. The adult F_1 males were isolated and crossed with *al dp b cn* females to obtain TC_1 progeny which was screened for phenotype and sex. The protocol of the whole experiment is given in **fig. 3.1**.

Fig. 3.1. PROTOCOL OF THE EXPERIMENT

Organism : *Drosophila melanogaster*
Probe : Maleic hydrazide



3.8 Preparation of Temporary Smears of Salivary Gland cells

The late third instar larvae which were crawling over the walls of the vial and had become slightly inactivated were selected for dissecting out the salivary glands. The dissection was carried out under an Olympus dissecting stereobinocular microscope by placing a larva in a drop of saline (0.7% NaCl) solution, in a cavity slide. The larva was held tightly with a fine stout needle kept in the middle. With the help of another fine needle placed at the anterior end of the larva the salivery glands along with the head were pulled out. The dissected glands were cleaned by removing the surrounding fat bodies.

The glands were hydrolysed in N/10 HCl for four to five minutes. The hydrolysed glands were kept in acetocarmine stain for ten to twelve mintues. The stained glands were squashed under a cover slip (covered over by a number of folds of the blotting paper) by applying a gentle and uniform thumb pressure. The coverslip was sealed with vasseline to aviod drying up of the preparation. The temporary smears were stored in a Coplin jar saturated with alcohol vapours, in a refrigerator, at 4°C. The slides were screened under Olympus binocular light microscope at 1,000X magnification for the presence of chromosomal aberrations.

3.9 Identification of Chromosomal Aberrations

The characteristic landmarks and freetips of the salivary gland chromosomes were compared with the standard salivary chromosome map of Bridges (1936) in order to pin point the breakage-union points of each aberration scored.

3.10 Statistical Analysis

(i) In order to teat the difference in the proportions of the recombinants in the pregenies of untreated and MH-treated flies, Z-test was used (Gupta, 1980).

(ii) To determine whether a particular progeny fits into a particular ratio Chi Square (χ^2) test was used (Gupta, 1980).

CHAPTER IV

RESULTS

4.1 Introduction of Recombination in F_1 Males of *D. melanogaster*

The pooled TC_1 pragenies (males and females) of all the untreated (Control) and 0.5% Maleic hydrazide (MH)-treated F_1(+/*al dp b cn*) males of *D. melanogaster* are given in table 4.1.

In the control expepriment, 60 F_1 males test crossed produced a pooled TC_1 progeny of 8001 flies comprising of 4033 males and 3968 females. In this pooled test cross progeny, 11 recombinant comprising of 3(1 *al* and 2 *cn*) males and 8 (2 *dp cn*, 4 *al* and 2 *cn*) females were obtained. Out of 90 MH-treated F_1 males test crossed, a pooled TC_1 progeny of 11843 flies comprising of 5949 males and 5894 females was recovered. In this pooled test cross progeny, 211 recombinants comprising of 84 (54 *b cn*, 13 *al dp*, 8 *al b cn* and 9 *dp*) males and 127 (61 *b cn*, 24 *al dp*, 19 *al b cn*, 9 *dp* and 14 *cn*) females were obtained. In TC_1 progeny of MH-treated F_1 males, *b cn* was the most and *al dp* was the next most frequent recombinant phenotype observed.

Out of 60 untreated and 90 MH-treated F_1 males test crossed only 6 (10.0%) and 23 (25.5%) produced recombinants, respectively. The average number of TC_1 recombinants produced per F_1 male (considering only those F_1 males that produced

Table 4.1. Pooled TC$_1$ progenies produced by untreated and 0.5% MH-treated F$_1$ **(+/*al dp b cn*) males of *D. melanogaster*.**

Phenotype	TC$_1$ Progeny					
	Control (60)			0.5% MH (90)		
	Males	Females	Total	Males	Females	Total
Wild type	3430	3404	6834	5015	4716	9731
al dp b cn	600	556	1156	850	1051	1901
al b cn	0	0	0	8	19	27
al dp	0	0	0	13	24	37
b cn	0	0	0	54	61	115
dp cn	0	2	2	0	0	0
al	1	4	5	0	0	0
dp	0	0	0	9	9	18
cn	2	2	4	0	14	14
Total recombinants	3	8	11	84	127	211
Total progeny	4033	3968	8001	5949	5894	11843

Figures within parenthesis indicate number of F$_1$ males test crossed.

recombinants) was 1.83 in the control and 3.65 in the MH experiments.

4.2 Per cent Recombinant Flies in TC_1 Progeny

The per cent recombinant flies scored in the TC_1 progenies of twenty-three MH-treated F_1 males of *D. melanogaster* is given in **table 4.2.** The nomenclature of the F_1 males is as follows: Control 60 F_1 males were numbered as C1, C2.......and C60. Similarly, the 90 MH-treated F_1 males were numbered as MH1, MH2.... and MH90. Here "C" refer to the control and "MH" refers to the Maleic Hydrazide experiment.

In the control experiment, the least frequency (0.85%) of male recombination was observed in TC_1 progeny of F_1 male C1; the highest frequency (1.89%) being in C11. The least frequency (2.325%) of male recombination in MH experiment was observed in TC_1 progeny of F_1 male MH78 and highest frequency (15.85%) being in MH85, which was closely followed by F_1 male MH38 (15.00%).

The recombinant *b cn* was produced in individual TC_1 progenies of 20 F_1 males (MH4, MH7, MH10, MH24, MH26, MH38, MH44, MH48, MH59, MH60, MH68, MH72, MH73, MH74, MH77, MH78, MH81, MH84, MH85 and MH90). The recombinant *al dp* was obtained in TC_1 progenies of 11 different F_1 males (MH22, MH24, MH29, MH38, MH44, MH68, MH72, MH74, MH77, MH81 and MH84). The recombinant *al b cn* was obtained in TC_1 progenies of 6 F_1 males (MH38, MH60, MH74, MH85, MH88 and MH90). The recombinant *cn*

Table 4.2. Per cent recombinant flies scored in TC$_1$ progenies of 6 untreated and 23 0.5% MH-treated F$_1$ (+/*al dp b cn*) males of *D. melanogaster*.

Designation of F$_1$ adults male	% recombinant flies in TC$_1$ progency	Phenotype and no.of recombinants	Size of TC$_1$ progency
1	2	3	4
CONTROL			
C1	0.85	*cn (1)*	117
C2	1.30	*dp cn (2)*	153
C4	0.92	*cn (1)*	108
C11	1.89	*al (3)*	158
C16	1.38	*cn (2)*	144
C20	1.60	*al (2)*	125
0.5% MH			
MH4	4.30	*b cn* (4)	93
MH7	3.00	*b cn* (4)	130
MH10	3.50	*b cn* (4)	114
MH22	3.05	*al dp* (4)	113
MH24	7.50	*b cn* (5) *al dp* (4)	120
MH26	6.43	*b cn* (9)	140
MH29	4.03	*al dp* (6)	149
MH38	15.00	*b cn* (1) *al dp* (5) *dp* (7) *al b ch* (2)	100
MH44	6.72	*b cn* (6) *al dp* (3)	134
MH48	3.20	*b cn* (4)	125
MH59	9.90	*b cn* (10) *cn* (2)	132
MH60	12.78	*b cn* (12) *al b cn* (5)	133

Contd...

Table 4.2 Continued...

1	2	3	4
MH68	12.95	*b cn* (14) *al dp* (3) *dp* (1)	139
MH72	9.09	*al dp* (2) *b cn* (5) *cn* (4)	121
MH73	10.60	*b cn* (8) *cn* (3)	103
MH74	14.28	*b cn* (10) *al dp* (4) *al b cn* (1) *dp* (2)	119
MH77	9.52	*b cn* (4) *al dp* (2) *a* (4)	105
MH78	2.33	*b cn* (2)	86
MH81	4.60	*b cn* (1) *al dp* (2) *cn* (1)	86
MH84	5.50	*b cn* (4) *al dp* (2)	108
MH85	15.85	*al b cn* (5) *dp* (8)	82
MH88	5.30	*al b cn* (7)	132
MH90	8.98	*b cn* (8) *al b cn*(7)	167

was produced in TC_1 progenies of 4 F_1 males, MH59, MH72, MH73 and MH81. The recombinant *dp* was yielded by 4 F_1 males (MH38, MH68, MH74 and MH85) in their reepective TC_1 progenies.

The number of recombinants of a particular phenotype recovered in TC_1 progenies of 6 untreated and 23 MH-treated F_1 males is also given in table 4.2. Size of TC_1 progeny produced by 6 untreated and 23 MH-treated F_1 males, recorded in table 4.2, ranges from 86 to 167.

4.3 Overall Per cent Recombination

The overall per cent recombination observed in *al-dp, dp-b* and *b-cn* regions in the pooled TC_1 progenies of control and MH-treated F_1 males of *D. melanogaster* is given in table 4.3. The Z-values obtained in different comparisons are also given in this table.

The per cent recombination observed separately or together in the three regions in MH-experiment was significantly higher over the control ($p<0.001$). The per cent male recombinantion noted in *al-dp* (0.087), *dp-b* (0.024) and *b-cn* (0.074) and overall per cent of male recombination (0.137) in the pooled TC_1 progenies of untreated F_1 males was insignificant. The per cent male recombination was highest in *dp-b* (1.661) followed by *al-dp* (0.372) and *b-cn* (0.011) regions. The overall per cent recombinants recorded in MH-experiment was 1.78.

Table 4.3. Per cent recombination observed and test of significance in *al-dp, dp-b* and *b-ch* regions of untreated and MH-treated F_1 (+/*al dp b cn*) males of *D. melanogaster*.

Region	Percent recombination		
	Control	MH	Z- Value
al-dp	0.087	0.372	45.69^{***}
dp-b	0.024	1.661	1175.70^{***}
b-cn	0.074	0.110	4620.00^{***}
Total	0.137	1.780	332.07^{***}

$^{***}p < 0.001$.

4.4 Segregation Patterns

Segregation patterens revealed in control and 0.5% MH experiments by F_1 (+/*al dp b cn*) males of *D. melanogaster* at the *al, dp, b* and *cn* loci are given in **table 4.4.** The χ^2 values are also given in this table.

In the control experiment, non-Mendelian segregation was observed at all the four loci studied ($p<0.001$). In MH experiment, segregation pattern was again found to be non-Mendelian ($p<0.001$). A common feature of all the loci showing distorted segregation in control and MH experiments was that this phenomenon was always accompanied by higher frequency of "wild type" alleles over their respective "mutant" alleles, when considered separately. Thi Chi-Square values are higher in MH-experiment as compared to control (Table 4.4) which revealed that non-Mendelian segregation patterens is more conspicuous in MH experiment than in the control.

4.5 Sex-Ratio

The number of male and female flies obtained in different phenotypic classes of the pooled TC_1 progenies produced by F_1 (+/*al dp b cn*) males in control and MH (0.5%) experiments, given in table 4.1, were tested for 1:1 ratio using χ^2 test. The χ^2 value thus obtained are given in **table 4.5.**

Table 4.4. Segregation patterns revealed in TC$_1$ progenies of untreated and MH-treated F$_1$ (+/*al dp b cn*) *D. melanogaster* males, at the four loci studied.

Locus	Control	0.5% MH
al	$al < +^{***}$ (2015.4)	$al < +^{***}$ (2643.5)
dp	$dp < +^{***}$ (2019.6)	$dp < +^{***}$ (2655.6)
b	$b < +^{***}$ (2022.5)	$b < +^{***}$ (7363.0)
cn	$cn < +^{***}$ (2014.0)	$cn < +^{***}$ (2522.0)

χ^2 values are given in paranthesis.

Table 4.5. Testing various phenotypes of pooled TC_1 progenies produced by untreated and MH-treated F_1 (+/*al dp b cn*) males of *D. melanogaster*.

TC_1 Phenotype	Control	0.5% MH
Wild type	M = F (0.05)	M > F(4.59)*
al dp b cn	M = F (0.84)	M > F(10.06)***
al b cn	NR	M = F(2.24)
al dp	NR	M = F(1.63)
b cn	NR	M = F(0.22)
dp cn	M = F (0.5)	NR
al	M = F (0.9)	NR
dp	NR	M = F (0.00)
cn	M = F (0.0)	M < F (7.0)**

NR = No recombinant ; M = Males; F = Females.

Within parenthesis are given the χ^2 values.

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

In control experiment, the numbers of TC_1 males and females obtained in the parental (wild type and *al dp b cn*) as well as in recombinant classes (*dp cn, al* and *cn*) recovered were not statistically different. However, in MH experiment, the number of TC_1 males in both parental classes were not in 1:1 ratio; in wild type the number of males were more than number of females ($p<0.05$). In *al dp b cn*, the number of males were less than the number of females ($p<0.001$). In 3 recombinant types (*al b cn, al dp* and *b cn*) the numbers of males and females were not different statisticaly. In recombinant type *cn*, the number of females was more than that of males ($p<0.01$).

4.6 Testing the Complementary Classes for 1:1 Ratio

The number of flies recovered in different complementary classes (data given in table 4.1) were compared using χ^2 test to see whether male recombination induced with MH in *D. melanogaster* was reciprocal or non-reciprocal in nature. When the two complementary classes appeared in 1:1 ratio, male recombination was regarded as 'reciprocal' but when they failed to appear in 1:1 ratio, male recombination was termed as 'non-reciprocal'. Summary of the analysis performed for testing the complementary calsses for 1:1 ratio in TC_1 progeny of F_1 (+/*al dp b cn*) *D. melanogaster* males in control and 0.5% MH experiments alonghwith χ^2 values obtained, is given in **table 4.6**.

Table 4.6. Summary of χ^2 test in complementary classes for 1:1 ratio in TC_1 progenies produced by F_1 (+/*al dp b cn*) males of *D. melanogaster*.

Phenotype	Control	0.5% MH
Wild type vs *al dp b cn*	$>^{***}$ (2017.5)	$>^{***}$ (2635.0)
al vs *dp b cn*	= (1.0)	NR
al dp vs *b cn*	NR	$<^{***}$ (18.9)
al dp b vs *cn*	= (2.0)	$<^{***}$ (7.0)
al b cn vs *dp*	NR	= (0.9)
al dp cn vs *b*	NR	NR
al cn vs *dp b*	NR	NR
al b vs *dp cn*	= (1.0)	NR

NR - No recombinants; $^{***}P<0.001$;

χ^2 values are given in parenthesis.

Considering the two parental classes first, the wild type phenotype was found to be predominant over quadruple mutant (*al do b cn)* in control ($p<0.001$) as well as in 0.5% MH experiment ($p<0.001$). The three pairs of complementary recombinant phenotypes, *al* Vs *dp b cn; al dp b* Vs *cn*; and *al b* vs *dp cn*, were observed in equal frequency in the control experiment (table 4.6). In the MH experiment, one complementary pair of recombinant phenotypes *al dp* Vs *b cn* showed the number of flies of the latter phenotype to be in majority over the former ($p<0.001$). The same was observed in another pair of complementary classes, *al dp b* Vs *cn*, ($p<0.001$). For the complementary pair of recombinant phenotype *al b cn* vs *dp*, equal frequency of individuals was observed statistically.

4.7 Verification of TC_1 Recombinant Males

The TC_1 recombinant males isolated in the present study are designated as C04.32...... and so on in control, and MH04.51...... and so on in MH experiments. The numercial number before the decimal represents the F_1 male and that followed by the decimal represents serial number at which the particular recombinant is listed in data sheet of a particular TC_1 progeny of an F_1 male.

All the TC_1 recombinant males of *D. melanogaster* recovered in the control and 0.5% MH experiments were verified genetypically/phenotypically by further test crossing them with *al dp b cn* females. A TC_1 recombinant male was considered as verified when it produced recombinant type flies in addition to the quadruple recessive type in the TC_2 progeny. The percentage of individuals showing recombinant phenotype in TC_2 progeny of a particular TC_1 recombinant male is given in **table 4.7.**

The least per cent of recombinant type flies observed was 5.50 in TC_2 progeny of TC_1 recombinant male MH38.34 (*dp*) and the highest being 84.37 in TC_2 progeny of TC_1 male MH74.73 (*b cn*). The average per cent of recombinant typed flies in 84 TC_2 progenies of TC_1 male recombinants was came out to be 47.95 (i.e. close to 50%) in MH experiment. The number of recombinant and *al dp b cn* types flied recovered in TC_2 progeny of a TC_1 recombinant male were tested for expected 1:1 ratio using χ^2 test. The χ^2 values obtained are also given in table 4.7. The analysis shows that the two phenotypes in TC_2 progenies of 87 (3 control and 84 MH-induced) TC_1 males, 48 (55.17%) appeared in 1:1 ratio. The per cent recombinant flies in different TC_2 progenies of this category ranged from 41.0 to 67.0. In the remaining 39 (44.82%) TC_2 progenies, the recombinant and *al dp b cn* phenotypes did not appear in 1:1 ration; ten (11.49%) of these progenies showed per cent recombinant type flies from 39.0 to 41.0 and 68.0 to 73.0 and the level of significance was $p<0.05$. The per cent recombinant type flies in fifteen (17.24%) progenies ranged from 28.0 to 39.0 and 73 to 80.0 per cent and the level of significance was

Table 4.7. Percent recombinant flies obtained in TC_2 progenies of untreated and 0.5% MH-induced TC_1 male recombinants of *D. melanogester*.

Designation of TC_1 male recombinants	Phenotype	% recombinant type flies in TC_2 progeny
1	2	3
CONTROL		
■C04.32	*cn*	48.27
■C16.38	*cn*	32.80**
C20.15	*al*	14.06***
0.5% MH		
■MH04.51	*b cn*	31.25**
■MH04.52	*b cn*	42.00
■MH07.28	*b cn*	40.23
MH07.29	*b cn*	53.84
MH10.09	*b cn*	42.00
MH10.10	*al b cn*	43.00
MH12.45	*al dp*	38.86
MH12.48	*al dp*	34.32**
■MH22.67	*al dp*	51.26
MH24.58	*b cn*	32.05**
■MH24.59	*al dp*	21.00***
MH26.61	*b cn*	50.06
MH26.63	*b cn*	39.88*
MH26.64	*b cn*	21.69***
MH29.59	*al dp*	30.36**
■MH29.60	*al dp*	31.40**
MH38.33	*dp*	10.30***
MH38.34	*dp*	5.50***
MH38.35	*dp*	5.86***
MH38.51	*al b cn*	42.61
MH38.52	*al b cn*	40.65
■MH44.64	*b cn*	58.83
■MH44.65	*b cn*	54.58

Continued...

Table 4.7. Continued...

1	2	3
MH44.71	*al dp*	38.89^{*}
MH48.66	*al dp*	50.00
MH48.67	*b cn*	61.39
MH59.67	*b cn*	60.40
■MH59.68	*b cn*	51.11
■MH59.71	*b cn*	51.11
MH59.72	*b cn*	52.22
MH59.73	*b cn*	52.22
MH60.34	*b cn*	54.65
MH60.35	*b cn*	51.00
MH60.36	*b cn*	61.37
MH60.37	*b cn*	68.40^{*}
MH60.38	*b cn*	65.37
■MH60.49	*al b cn*	40.00^{*}
■MH68.68	*b cn*	60.00
■MH68.69	*b cn*	61.55
MH68.70	*b cn*	61.29
MH68.71	*b cn*	51.10
MH68.72	*b cn*	52.08
■MH68.73	*b cn*	59.90
■MH68.74	*b cn*	50.00
■MH68.81	*al dp*	30.66^{**}
■MH68.82	*al dp*	29.56^{**}
■MH72.48	*al dp*	28.88^{**}
■MH72.49	*b cn*	51.65
■MH72.50	*b cn*	52.66
■MH72.51	*b cn*	68.87^{*}
■MH72.52	*b cn*	60.00
■MH73.14	*b cn*	63.34
■MH73.15	*b cn*	62.59
■MH73.16	*b cn*	49.90
■MH73.17	*b cn*	39.81^{*}
■MH74.58	*al dp*	21.56^{***}

Continued...

Table 4.7. Continued...

1	2	3
■MH74.59	*al b cn*	40.26^{*}
■MH74.72	*b cn*	50.77
■MH74.73	*b cn*	84.37^{***}
MH74.74	*b cn*	75.90^{**}
■MH74.75	*b cn*	58.33
■MH74.81	*b cn*	68.11^{*}
MH74.82	*b cn*	64.59
MH74.88	*dp*	11.53^{***}
■MH74.89	*dp*	8.20^{***}
MH77.22	*b cn*	57.20
MH77.23	*b cn*	57.40
■MH77.44	*b cn*	63.46
■MH77.45	*b cn*	76.40^{**}
■MH78.65	*b cn*	65.62
■MH84.30	*al dp*	54.58
■MH84.31	*b cn*	65.62
■MH84.32	*b cn*	74.35^{**}
■MH85.18	*al b cn*	37.66^{**}
■MH85.31	*dp*	17.02^{***}
■MH85.32	*dp*	16.20^{***}
■MH85.33	*dp*	9.67^{***}
■MH85.34	*dp*	12.50^{***}
■MH88.73	*al b cn*	58.33
■MH88.75	*al b cn*	60.45
■MH90.47	*b cn*	80.85^{***}
■MH90.48	*b cn*	74.41^{**}
■MH90.49	*b cn*	69.86^{*}
■MH90.50	*b cn*	80.00^{**}
	Average	47.32

■ The TC_1 male recombinants which were used to construct homozygous male recombinant lines.

The TC_2 progenies in which the recombinant and *al dp b cn* type flies did not appear in 1:1 ratio have been indicated as $^{*}p < 0.01$; $^{**}p < 0.01$; $^{***}p < 0.001$.

$p<0.001$ and this value in fourteen (16.09%) progenies ranged from 5.0 to 22.0 and 80.0 to 85.0 and the level of significance was $p<0.001$.

Bar diagram of frequency distribution of per cent recombinant type flies recovered in TC_2 progenies of 87 (3 control and 84 MH-induced) TC_1 recombinant males is given in **fig. 4.1.** A study of this bar diagram suggests almost normal distribution in respect of per cent recombinant flies recovered in TC_2 progenies of these TC_1 recombinant males. Further, sixty-seven (77.0%) of the TC_2 progenies have yielded per cent recombinant type flies from 30-75%, the remaining twenty (23%), TC_2 progenies fall in the two extremities.

4.8 Construction of Homozygons Male Recombinant Lines

Through several full-sib matings of TC_2 males and females having recombinant phenotype same as their TC_1 recombinant male (father), a total of 52 (2 from control and 50 from MH expriment) homozygouns male recombinant lines (HMRLs) were constructed. The designation and phenotypes of these 52 HMRLS are given in **table 4.8.** Salivary chromosome examination and male recombination studies were conducted on these HMRLS. Table 4.8 gives the information about the HMRLs in which the study of chromosomal aberrations and/or male recombination was done. Out of 52 HMRLS constructed, in only 25 HMRLs it was possible to do both the studies. In other 20

Fig 4.1. Bar diagram of frequency distribution of per cent recombinant type flies recovered in progenies of recombinant males

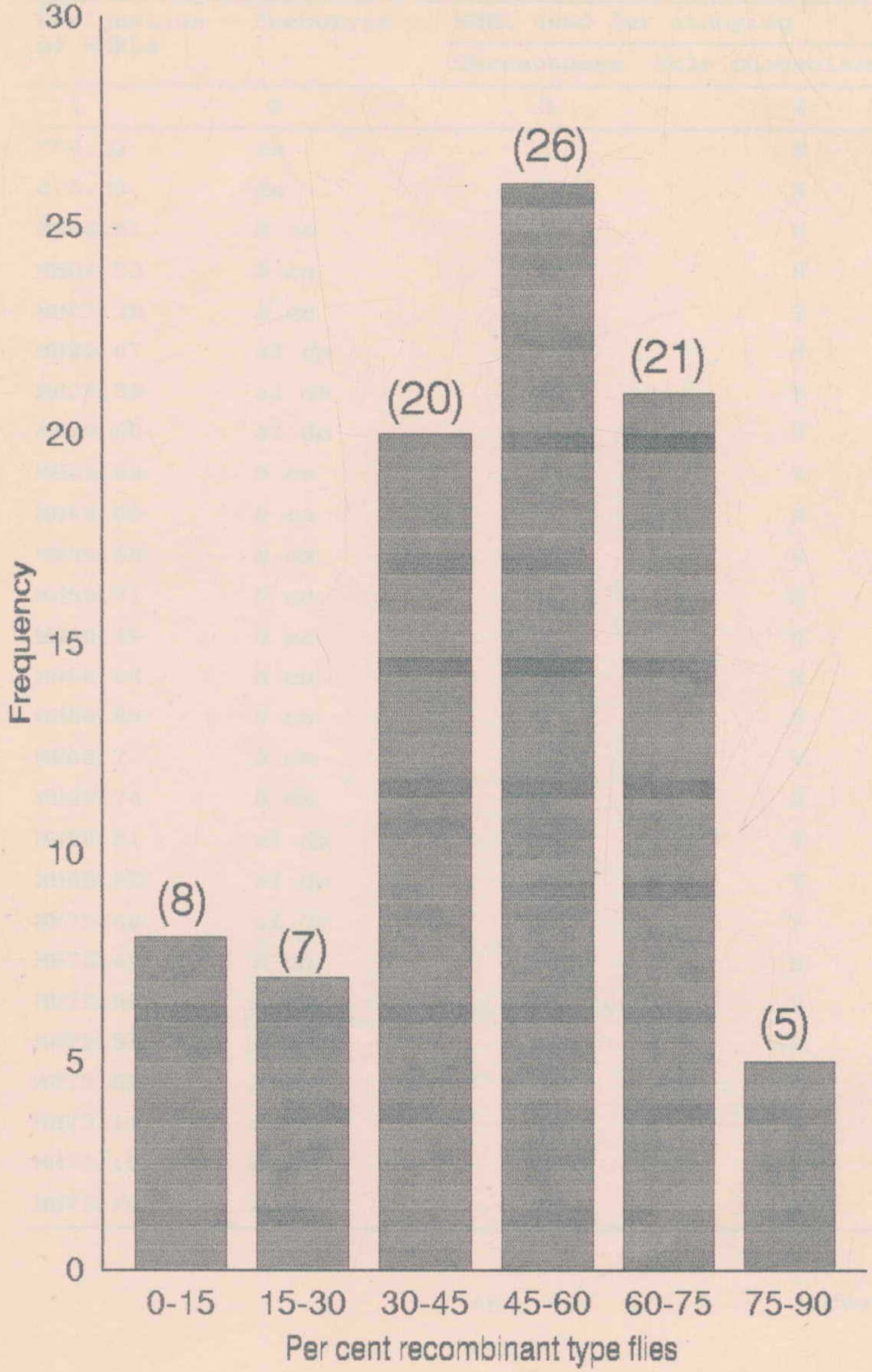


Table 4.8. **Information about the HMRLs used for chromosome and male recombination studies in *D. melanogaster*.**

Designation of HMRLs	Phenotype	HMRL used for studying	
		Chromosomes	Male recombinantion
1	2	3	4
C04.32	*cn*	Y	N
C16.38	*cn*	Y	N
MH04.51	*b cn*	Y	N
MH04.52	*b cn*	N	N
MH07.28	*b cn*	Y	Y
MH22.67	*al dp*	N	N
MH24.59	*al dp*	N	N
MH29.60	*al dp*	N	N
MH44.64	*b cn*	Y	Y
MH44.65	*b cn*	Y	N
MH59.68	*b cn*	Y	N
MH59.71	*b cn*	N	N
MH60.49	*b cn*	Y	N
MH68.68	*b cn*	Y	N
MH68.69	*b cn*	Y	N
MH68.73	*b cn*	Y	Y
MH68.74	*b cn*	Y	N
MH68.81	*al dp*	Y	Y
MH68.82	*al dp*	Y	Y
MH72.48	*al dp*	Y	Y
MH72.49	*b cn*	N	N
MH72.50	*b cn*	Y	Y
MH72.51	*b cn*	Y	Y
MH72.52	*b cn*	Y	Y
MH73.14	*b cn*	Y	N
MH73.15	*b cn*	Y	N
MH73.16	*b cn*	Y	N

Contd...

Table 4.8 Continued...

1	2	3	4
MH73.17	*b cn*	Y	N
MH74.58	*al dp*	Y	N
MH74.59	*al b cn*	Y	Y
MH74.72	*b cn*	Y	N
MH74.73	*b cn*	Y	Y
MH74.75	*b cn*	Y	Y
MH74.81	*b cn*	Y	Y
MH74.89	*dp*	Y	Y
MH77.44	*b cn*	Y	Y
MH77.45	*b cn*	Y	Y
MH78.65	*b cn*	Y	N
MH84.30	*al dp*	Y	Y
MH84.31	*b cn*	Y	Y
MH84.32	*b cn*	Y	Y
MH85.18	*al b cn*	Y	N
MH85.31	*dp*	Y	Y
MH85.32	*dp*	Y	Y
MH85.33	*dp*	N	N
MH85.34	*dp*	Y	N
MH88.73	*al b cn*	Y	N
MH88.75	*al b cn*	Y	N
MH90.47	*b cn*	Y	N
MH90.48	*b cn*	Y	Y
MH90.49	*b cn*	Y	Y
MH90.50	*b cn*	Y	Y

Y = Yes; N = No.

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HMLRs neither aspect could be studied as these lines could not be maintained in the labortary.

4.9 Study of Salivary Chromosomes

The numbers of larvae disected, cells scored and aberrations detected in F_1, TC_1, TC_2, FS, FS_2, FS_3 and F1 generation larvae produced in the crosses HMRLs X complementary phenotype, or its reciprocal, are given in **table 4.9**. The nature and breakage-union points involved in different chromosomal aberrations are given in **table 4.10**. The observations on salivary chromosomes during these different generations are described in the following paragrphs.

4.9.1 F_1 larvea

In order to test the ability of MH to induce chromosomal aberrations in *D. melanogaster*, the F_1 larvae were randomly selected for examination of their salivary chromosomes. In 2256 cells examined from 12 F_1 larvae of control experiment no aberration was detected. The number of larvae studied in 0.5% MH experiment was 16. Three aberrations were detected in total of 3999 cells scored. The number of aberrations per F_1 larva was 0.187 and number of aberrations per hundred cells was 0.075. From MH-treated F_1 larva MH4, one inversion, 2R(50A-52F), was detected. In F_1 larvae MH7 one inversion, 2L(32F-36D), and in MH10, an inversion, X(5A-6F), was observed (Table 4.10).

Table 4.9. Number of larvae dissected, cells scored and aberrations detected in several *D. melanegaster* generations of control and 0.5% MH experiments.

Generations	Larvae dissected		Cells scored		Aberrations detected	
	Control	MH	Control	MH	Control	MH
F_1	12	16	2256	3999	0	3
TC_1	40	46	14030	15000	0	3
TC_2	15	184	2976	24757	0	6
FS_1	8	43	1356	10040	0	2
FS_2	8	44	1400	13567	0	1
FS_3	6	19	1138	4071	0	0
□ F_1	0	88	0	17055	0	0

□ These F_1 individuals were obtained from the crosses: HMRL X complementary phenotype, or its reciprocal.

Table 4.10. The nature and description of the chromosomal aberrations detected in the F_1 (+/*al dp b cn*) larvae of *D. melanogaster*.

Larvae designation	Nature of aberration	Chromosome and breakage-union points involved
F_1		
MH4	Inversion	2R(50A-52F)
MH7	Inversion	2L(32F-36D)
MH10	Inversion	X(5A-6F)
TC_1		
MH7.28	i) Inversion	2L(22D-26A)
	ii) Inversion	2L(21E-23F)
MH38.33	Inversion	3L(69F-71A)
TC_2		
MH68.81	Deletion	2L(34D-38D)
MH68.73	Inversion	2L(23F-26A)
MH74.59	Inversion	2L(24A-26B)
MH74.51	Deletion	2L(32A-34F)
MH78.65	Inversion	3L(67E-69D)
MH74.82	Inversion	2L(24A-26B)
FS_1		
MH74.59	Inversion	2L(36D-38F)
MH74.82	Inversion	2L(24A-26B)
FS_2		
MH72.50	Inversion	3L(67D-69F)

4.9.2 TC_1 larvae

In 14030 cells examined from 40 TC_1 larvae sampled from control experiment no aberration was observed. In MH experiment, out of 15,000 cells examined from 46 larvae, 3 aberrations were detected. Two inversions, 2L(22D-26A) and 2L(21E-23F) were observed in two different cells of the same TC_1 larva MH7.28. One inversion, 3L(69F-71A), was observed in TC_1 larva MH38.33. The number of aberrations detected per TC_1 larva was 0.06 and this value per hundred cells was 0.02.

4.9.3 TC_2 larvae

In control experiment, out of 2976 cells screened from 15 TC_2 larvae no aberration was observed whereas out of 184 TC_2 larvae dissected from MH expriment 6 aberrations were detected from 24757 cells scored. Two deletions, namely, 2L(34D-38D) and 2L(32A-34F) detected in two TC_2 larvae that had descended from two different TC_1 recombinant males MH68.81 and MH74.51, respectively. Four inversions in 4 different larvae were detected. These inversions, namely, 2L(23F-26A), 2L(24A-26B), 3L(67E-69D) and 2L(24A-26B) were observed in TC_2 larvae that had descended from four different TC_1 recombinant males MH68.73, MH74.59, MH78.65 and MH74.82, respectively. The number of aberrations per TC_2 larvae was 0.0326 and that per hundred cells scored was 0.024.

4.9.4 FS_1 larvae

In 1356 cells examined from 8 FS_1 larvae from control expriment, no aberration was observed. In MH experiment, out of 10040 cells scored from 43 FS_1 larvae, two inversions were detected. An inversion, 2L(36D-38F), was detected in FS_1 larva that had descended from TC_1 recombinant male MH74.59. Another inversion, 2L(24A-26B), was observed in FS_1 larva that had descended from TC_1 recombinant male MH74.82. The number of aberrations per FS_1 larva and that per hundred cells scored was 0.046 and 0.019, respectively.

4.9.5 FS_2 larvae

In 1400 cells screened from FS_2 larvae from control experiment no aberration was detected. In MH experiment, out of 13567 cells examined from 44 FS_2 larvae. Only one inversion, 3L(67D-69F), was observed in FS_2 larva that had descended from TC_1 recombinant male MH72.50. The number of aberrations observed per FS_2 larva was 0.023 and this value per hundred cells scored was 0.007.

4.9.6 FS_3 larvae

In control experiment, the number of larvae studied was 6. A total of 1138 cells were scored and no aberration was detected. As many as 4071 cells were scored from 19 FS_3 larvae from MH experiment where again no aberration was detected.

4.9.7 F_1 larvae of crosses : HMR x complementary phenotype, or it reciprocal

The number of larvae studied in MH experiment from these crosse was 88 and 17055 cells were screened. No aberration was detected.

The chromosomal aberrations detected, in salivary chromosomes sampled from different generations, have been mapped on the cytogenetic map of King (1975) and is shown as fig. 4.2.

4.10 Male recombination Studies in F_1 Males yielded by crossing HMRL x Complemantry Phenotype or its Reciprocal

Out of 50 HMRLs constructed (listed in table 4.8), the females from 25 lines were crossed with males from respective complementary stocks to obtain F_1 generation. These HMRLs are designated by prefixing "G" indicating name of the author. Reciprocal crosses were also conducted. Out of 25 HMRLs studied, in reciprocal crosses for their potential to produce male recombination, 16 had *b cn*, 4 *al dp*; 3 *dp* and 2 *al b cn* phenotype. The F_1 males thus obtained were test crossed with *al dp b cn* females. The per cent male recombination observed in different TC_1 progenies is given in table 4.11.

4.10.1 HMRLs of recombinants *b cn*

Out of 16 pairs of reciprocal crosses made using *b cn* HMRLs, male recombination was observed in 13 (GM07.28,

EXPLANATION OF FIG. 4.2

The chromosomal aberrations detected, in salivery chromosomes from the larvae sampled from different generations, maped on cytogenetic map of King (1975). Chromosomes on which the aberrations are located and their breakage-union points are given in table 4.10. The aberrations detected in different generations are shown in fig. 4.2. in different colours as mentioned below :

Generation	Colour
F_1	Black
TC_1	Red
TC_2	Brown
FS_1	Green
FS_2	Voilet

Table 4.11. **Per cent of recombinants obtained in the progenies of crosses *al dp b cn* x F_1 (homozygous male recombinant line x complemetaary phenotype, or its reciprocal) in *D. melanogaster*.**

Female and male crossed to obtain F_1 males	Recombinants in the test cross progeny	
	Per cent	Phenotypes
1	2	3
GM 07.28 *b cn* x *al dp*	15.50	*al dp b cn*
al dp x GM 07.28 *b cn*	12.00	*al dp b cn*
GM 44.64 *b cn* x *al dp*	21.50	*al b cn, al dp b cn*
al dp x GM 44.64 *b cn*	8.40	*al dp b cn*
GM 68.73 *b cn* X *al dp*	10.80	*al b cn*
al dp x GM 07.28 *b cn*	12.90	*al dp cn*
GM 68.81 *al dp* x *b cn*	23.00	*al dp b cn*, +++, *dp*
b cn x GM 68.81 *al dp*	0.00	NR
GM 68.82 *al dp* x *b cn*	21.25	*al dp b cn*, +++, *dp*
b cn x GM 68.82 *al dp*	3.63	*al dp b cn*
GM 72.48 *al dp* x *b cn*	23.10	*al dp b cn*, ++++
b cn x GM 72.48 *al dp*	13.33	*al dp b cn*
GM 72.50 *b cn* x *al dp*	12.54	*al dp b cn*
al dp x GM 72.50 *b cn*	8.76	*al dp cn*
GM 72.51 *b cn* x *al dp*	29.50	*al dp b cn*
al dp x GM 72.51 *b cn*	0.00	NR
GM 72.52 *b cn* x *al dp*	24.30	*al dp b cn*
al dp x GM 72.52 *b cn*	21.0	*al dp cn*
GM 74.59 *al b cn* x *dp*	5.49	*dp cn*
dp x GM 74.59 *al b cn*	2.19	*dp cn*

Contd...

Table 4.11 Continued...

1	2	3
GM 74.73 *b cn* x *al dp*	17.54	*al dp b cn*
al dp x GM 74.73 *b cn*	20.67	*al b cn, al dp b cn*
GM 74.75 *b cn* x *al dp*	19.67	*al b cn, al dp b cn*
al dp x GM 74.75 *b cn*	21.57	*al b cn, al dp b cn*
GM 74.81 *b cn* x *al dp*	27.53	*al dp b cn, al b cn*
al dp x GM 74.81 *b cn*	18.55	*al b cn*
GM 74.89 *dp* x *al b cn*	6.50	*dp b cn*, ++++
al b cn x GM 74.89 *dp*	3.21	*al dp*, ++++
GM 77.44 *b cn* x *al dp*	25.26	*al b cn*
al dp x GM 77.44 *b cn*	20.00	*al b cn, al dp b cn*
GM 77.45 *b cn* x *al dp*	21.12	*al b cn, al dp b cn*
al dp x GM 77.45 *b cn*	19.09	*al dp b cn*
GM 84.30 *al dp* x *b cn*	12.75	*al dp b cn*, ++++
b cn x GM 84.30 *al dp*	0.00	NR
GM 84.31 *b cn* x *al dp*	00.00	NR
al dp x GM 84.31 *b cn*	00.00	NR
GM 84.32 *b cn* x *al dp*	16.21	*al b cn*
al dp x GM 84.32 *b cn*	00.00	NR
GM 85.31 *dp* x *al b cn*	4.05	++++, *al dp cn*
al b cn x GM 85.31 *dp*	00.00	NR
GM 85.32 *dp* x *al b cn*	00.00	NR
al b cn x GM 85.32 *dp*	00.00	NR
GM 88.73 *al b cn* x *dp*	3.16	*b cn*
dp x GM 88.73 *al b cn*	1.28	*al dp b cn*

Contd...

Table 4.11 Continued...

1	2	3
GM 90.48 *b cn* x *al dp*	20.46	*al b cn*
al dp x GM 90.48 *b cn*	18.88	*al dp b cn, al b cn*
GM 90.49 *b cn* x *al dp*	21.67	*al dp cn, al dp b cn*
al dp x GM 90.49 *b cn*	15.66	*al b cn*
GM 90.50 *b cn* x *al dp*	23.15	*al b cn*
al dp x GM 90.50 *b cn*	7.28	*al b cn*

NR = No recombinant recovered.

GM44.64, GM68.73, GM72.50, GM72.52, GM74.73, GM74.75, GM74.81, GM77.44, GM77.45, GM90.48, GM90.49 and GM90.50) pairs. In 2 pairs of reciporal crosses involving HMRLs GM72.51 and GM84.32, male recombination was not observed when the F_1 males were produced using HMRLs as female parent but when F_1 males were produced using HMRLs as female parent, male recombination was observed. When male recombination poltential was studied in HMRLs GM84.31, recombinants did not appear in either of the reciprocal crosses.

4.10.2 HMRLs of recombinants *al dp*

Only 4 HRMLs of phenotype *al dp* were studied for male recombination, in reciprocal crosses. In two HMRL, GM68.82 and GM72.48, male recombination was observed in all the cases. In other two sets of reciprocal crosses involving HMRLs GM68.81 and GM85.31, male recombination was observed only in the crosses where F_1 male was produced using HMRLs as female parent.

4.10.3 HMRLs of recombinants *dp*

Three *dp* HMRLS were studied for their potential to produce male recombination. In the reciprocal crosses involving HMRL GM74.89, male recombination was observed. In another HMRL, GM85.31, male recombination was observed only in the cross where F_1 male was produced using HMRL as a female parent. In HMRL GM85.32, male recombination was noted in niether of the reciprocal crosses.

4.10.4 HMRLs of recombinants *al b cn*

Two HMRLs (GM74.59 and GM88.72) of phenotype *al b cn* were studied for male recombination potential and by conducting reciprocal crosses. Male recombination was seen in reciprocal crosses of both these HMRLs.

The overall per cent male recombination observed in F_1 males produced when HMRLs were used as female parent was compared with that when F_1 male was produced using HMRLs as male parent. In the former type of crosses, the least frequency (3.16%) of male recombination was observed in case of HMRL GM88.73 of phenotype *al b cn*. And the highest frequency (29.50%) of male recombination was obtained in case of HMRL GM72.51 of phenotype *b cn*. In latter type of crosses, the minimum frequency (2.10%) of male recombination was observed for HMRL GM74.59 of phenotype *al b cn* and the maximum freqency (21.59%) was observed for HMRL GM74.75 of phenotype *b cn*. Thus the values depicting per cent male recombination in former type of crosses were higher than those in the latter type.

Out of a total of 50 reciprocal TC_1 progenies studied for the ability of 25 HMRLs of *D. melanogaster* to produce male recombination, 41 (82%) exhibited this potential.

CHAPTER V

DISCUSSION

5.1 Male Recombination in Labortary Strains

5.1.1 Overall frequency of male recombination

In *D. melanogaster* males spontaneous induction of recombination was reported in wild type labortary strains (Whittinghil and Lewis, 1961 ; Hannah-Alva, 1968; Hiraizumi *et al*; 1973) and in natural populations (Hiraizumi, 1971; Voelkar, 1974; Waddle and oster, 1974 Yamaguchi and Mukai, 1974; Kidwell and Kidwell, 1975a,b and 1975b; Woodruff and Thompson, 1977; Sved, 1978; Yannopoulous *et al*, 1981). No Spontaneous male recombination was observed in Oregon-K strain of *D. melanogaster* in *dumpty-black-cinnabar*, region by Miglani and Thapar (1982b), at 25°C. Singh (1992) did not observe spontaneous male recombination in *aristaless-dumpy-black-cinnabar* region of *D. melanogasater*. Howerver, Miglani and Mohindra (1986a) observed 0.267 per cent spontaneous recombination in males of oregon-K strain of *D. melanogaster* in *dumpy-black-cinnabar* region, at 25°C whereas Singh (1989) observed a higher frequency of spontaneous male recombination (0.74%) in the same region and still higher (0.96%) male recombination in *aristaless-dumpy-black* region in the same strains of *D. melanogaster* males. Preet (1989) also observed very high frequency (0.993%) of spontaneous male recombination in *aristalelss-dumpy-black-purple* region, at 25°C. In the present studies a low frequency (0.137%) of spontaneous male recombination in *aristaless-dumpy-black-cinnabar* region was, however, observed (Table 4.3).

5.1.2 Frequency of adults producing recombinants

About 35 per cent of untreated F_1 (+/*al dp b cn*) adults of *D. maleanogaster* revealed recombinants in their TC_1 progenies (Miglani and Mohindra, 1986a). Whereas many as 62.96 and 85.00 per cent untreated males of genotypes F_1 (+/*dp b cn*) and (+/*al dp b*), respectively, produced recombinants (Singh, 1989). Preet (1989) observed that 40.0 per cent of untreated F_1 (+/*al dp b pr*) males produced recombinants. According to Singh (1992), no F_1 (+/*al dp b cn*) male produced recombinants in the control expriment. In the present study, however, 10.0 per cent of the untreated F_1 (+/*al dp b cn*) males produced recombinants (Table 4.1).

5.1.3 Phenotypic spectrum of male recombinants

Miglani and Mohindra (1986a) observed in TC_1 progenies of untreated F_1 (+/*al dp b cn*) males of *D. melanogaster* (Oregon-K strain) only two recombinant phenotypes, *b cn* and *dp*; the former being 7-times more frequent than the latter. Singh (1989) reported *b cn* and *dp b* as the most common recombinant phenotypes produced by F_1 males of genotypes +/*dp b cn* and +/*al dp b*, respectively. In the control experiment conducted by Preet (1989), *al do* was the most and *b pr* the next most common recombinant phenotyp produced by F_1 (+/*al dp b pr*) males.

One thing common in all the cases where spontaneous male recombination was observed in that phenotypes resulting from male recombination in *dp-b* region was the most frequent in *D. melanogaster*. In the present study, three recombinant types, i.e.,*al, dp cn* and *cn* were obtained from untreated F_1 (+/*al dp b cn*) males of *D. melanogaster* of which *al* was the most whereas *dp ch* was the least frequent recombinant type observed.

5.1.4 Selective susceptibility of different regions

Different regions of *D. melanogaster* have been found to be differing for frequency of spontaneous male recombination in *D. melanogaster*. Thapar (1982) observed 37 times more male recombination in *dp-b* compared to *b cn* regions in *D. melanogaster*. Miglani and Mohindra (1986b) reported 0.133 per cent verified spontaneous male recombination in *dp-b* region but no male recombination was observed in *b-cn* region in untreated *D. melanogaster,* at 25°C. Singh (1989) reported 1.79 times higher spontaneous male recombination in *al-dp* than in *dp-b* region. Preet (1989) reported 4.88- and 1.2-times higher spontaneous male recombination in *dp-b* and *b-pr* regions, respectively, compared to *al-dp* region, in F_1 (+/*al dp b pr*) males of *D. melanogaster*.No spontaneous male recombination was found in the studies conducted by Singh (1992) in *al-dp, dp-b* and *b-cn* regions of *D. melanogaster*. In the present study,a very low frequency of spontaneous male recombination was observed in *al-dp* (0.087), *dp-b* (0.024) and *b-cn* (0.074) regions (Table 4.3) of *D. melanogaster*.

5.2 Male Recombination Induced by Maleic hydrazide

5.2.1 Overall per cent male recombination

The overall per cent male recombination in *D. melanogaster*, in the present study, with 0.5% maleic hydrazide (MH) was 1.78 (Table 4.3). This, perhaps, is the first time when recombinogenicity of MH has been reported. Thapar (1982) observed 3.355 per cent male recombination in F_1 (+/*al dp b cn*) males with 0.75% EMS when the treatment was given only during the second 32h period of larval life. Singh (1992), in a similar experiment, reported 1.331 while Miglani and Mohindra (1986a) reported 1.669 per cent recombination in F_1 (+/*al dp b cn*) males of *D. melanogaster* with the same dose of EMS. Sharma and Swaminathan (1968) injected 0.3% EMS solution intra-abdominally into adult F_1 (+/*al dp b cn)* *D. melanogaster* males and obtained 0.189 per cent recombination. Singh (1989) observed 1.05 per cent male recombination in *dp-b-cn* and 1.66 in *al-dp-b* region, when treatment was given with 0.5% HAS during the entire larval life of *D. melanogaster*. Preet (1989) observed the overalll per cent recombination with 0.25% fromaldehyde in F_1

(+/*al dp b pr*) males to be 0.489 whereas with 2.5% formaldehyde the same was 1.052. In this experiment also the treatment was given during the entire lalrval life of *D. melanogaster*.

Demopoulous *et al.* (1980) observed 0.26 per cent recombination when Canton/*dp b cn bw* males of *D. melanogaster* were treated with 20/ µg/ml bleomycin. Zimmering *et al.* (1966) observed 0.559 per cent recombination with 1,000r X-rays in F_1 (+/*dp b cn*) males. Thus, 0.5% MH, in the present study, was found to be superior over 20 µg/ml bleomyein (Demopoulos *et al.*, 1980); 1,000 r X-rays (Zimmering *et al.*,1966), 0.25% and 2.5% formaldehyde (Preet, 1989); 0.5% HAS (Singh, 1989); 0.25% formaldehyde (Sobels *et al.*, 1959); 0.3% EMS (Sharma and Swaminathan, 1968) and 0.75% EMS (Miglani and Thapar, 1983a; Miglani and Singh, 1992a), while 0.5% MH was found to exhibt inferiority over 0.75% EMS (Miglani) and Mohindra 1986a) for induction of male recombination in *D. melanogaster*.

5.2.2 Frequency of F_1 males producing recombinants

Miglani and Mohindra (1986a) observed that 5.05 per cent of F_1 (+/*al dp b cn*) males of *D. melanogaster* yielded recombinants in their TC_1 progenies when 0.75% EMS was fed to the devoloping larvae in their second 32h part. When 0.5% HAS was given to the F_1 males of the genotypes +/*dp b cn* and +/*al dp b* in their entire larval life, 91.25 and 96.0 per cent of them, respectively, produced recombinants in their TC_1 progenies (Singh, 1989).

Out of the F_1 (*+/al dp b pr*) males of *D. melanogaster* treated with 0.25% and 2.5% formaldehyde in their entire larval life, 29.03 per cent and 67.27 per cent males, respectively, yielded recombinants of chromosome 2 marker (Preet, 1989). Formaldehyde (0.25%) induced male recombination in chromosome 3 of *D. melanogaster* in 10 per cent of the males tested (Whiltinghill and Lewis 1961) while out of 17 X-irradiated (3,000 r) *Curly* males, 70.59 per cent produced recombinants in their progenies (Whittinghill, 1947).

In the present study, when the F_1 (*+/al dp b cn*) males were treated with 0.5% MH in their entire larval life, 23 (25.5%) out of 90 yielded recombinants. MH (0.5%) in the present sstudy, was found to be less effective than 3,000r X-rays, 0.75% EMS, 0.5% HAS and 2.5% formaldehyde in producing recombinants in higher frequency of *D. melanogaster* males. However, greater frequency of F_1 males produced recombinants with 0.5% MH, in the present experiment, than that with 0.25% formaldehyde (Whittinghill and Lewis, 1961).

5.2.3 Recombinant phenotypes

So far as phenotypic spectrum of recombinants is concerned, in the TC_1 progeny of *D. melanogaster* males treated with 0.5% HAS (Singh, 1989), 0.165% chloroquine phosphate (Miglani and Thapar, 1983a and 0.75% EMS (Miglani and Thapar, 1983; Miglani adn Mohindra, 1986a;) recombinant *b cn* was produced in the highest frequency. Singh (1992) recovered in *D. melanogaster* F_1 (+/*al dp b cn*) males, recombinant *al dp* in the highest frequency with 0.75% EMS. With 0.5% HAS, Singh (1989), also recovered in TC_1 progenies of F_1 (+/*al dp b*) males recombinant *al dp* in the highest frequency followed by *dp b*. The 0.25% and 2.5% formaldehyde-treated F_1 (+/*al dp b pr*) males of *D. melanogaster* yielded *al dp* as the most and *b pr* as the next most frequent type of recombinant in their TC_1 progenies (Preet, 1989). In the present study, 0.5% MH-treated F_1 (+/*al dp b cn*) males of *D. melanogaster* yielded *b cn* as the most and *al dp* as the next most prevalent recombinant type in their pooled TC_1 progeny (Table 4.1).

5.2.4 Differential susceptibility for male recombination in different regions

Different regions of chromosome 2 of *D. melanogaster* revealed differential susceptibility for induction of male recombination with maleic hydrazide, in the present study. The 0.5% MH induced 0.372 per cent male recombination in *al-dp*, 1.661 per cent in *dp-b* and 0.011 per cent in *b-cn* regions of *D. melanogaster*. Thus, on the present study, male recombination in *dp-b* region was found to be approximately 4.46- and 151.0-times higher as compared to *al-dp* and *b-cn* regions, respectively. With 0.75% EMS, Singh (1992) observed 0.116 per cent male recombination in *al-dp*; 1.351 per cent in *dp-b* and 0.019 per cent in *b-cn* regions of *D. melanogaster*; in this study also region *dp-b* showed highest susceptibility to EMS for inducing male recombinations in *D. melanogaster*.

With 0.5% HAS, Singh (1989) observed higher percentage of male recombination in *al-dp* (0.81) and *b-cn* (0.27%) than those with 0.5% MH in the present study, but in case of *dp-b* region, per cent recombination observed in present study (1.661%) is higher than that observed by Singh (1989) with HAS (0.90%) and that by Singh (1992) with EMS (1.351%).

With 0.25% formaldehyde, Preet (1989) observed lower percentages of recombination in *al-dp* (0.81) and *b-cn* (0.27%) than those with 0.5% MH in the present study, but in case of *dp-b* region, per cent recombination observed in the present study (1.661%) is higher than that with HAS (0.90%) and that by Singh (1992) with EMS (1.351).

With 0.25% formaldehyde, Preet (1989) observed lower percentage of recombination in *al-dp* (0.018%) and *dp-b* (0.887%) regions compared to those with 0.5% MH in the present study. Male recombination, observed with 0.5% MH in *dp-b* region, in the present study (1.661%) is higher than that reported by by Miglani and Thapar (1983b) (1.483%).

5.3 Segregation Distortion

According to Peacock and Erickson (1965), only half of the sperm of *Drosophila* males were capable of fertilizing female eggs and in case of segregation distortion, this half included mainly those gametes carrying the wild type chromosome. Woodruff and Lyman (1980) reported that in natural populations male recombination and meiotic derive were due to elements on the second chromosome and the region between *b* and *cn* contained distorter (SD) system. A second chromosome male recombination factor T-007 induced transmission ratio distortion (at its own expense) when heterozygous with many laboratory marker chromosomes. (Metthews, 1981)

In the present study, segregation distortion was observed at all the four loci, *al, dp, b* and *cn*, in test cross progenies of control and 0.5% MH experiments where "wild type" alleles were predominant over the corresponding "mutant type" alleles. Miglani and Mohindra (1986a) noted non-Mendelian segregation at *dp, b* and *cn* loci with EMS. Singh (1989) noted non-Mendelian segregation only at *dp* locus in control but Mendelian segregation at the *dp, b* and *cn* loci of HAS-treated F_1 males of *D. melanogaster*. A vast majority of cases (95%) studied by Preet (1989) exhibited segregation distortion at the loci *al, dp, b* and *pr* in different test crosse generation of control as well as formaldehyde (0.25% and 2.5%) expriments.

5.4 Sex - Ratio

Various parental and recombinant phenotypes of pooled TC_1 progeny were tested for 1:1 sex-ratio. In the TC_1

progeny produced by untreated F_1(+/*al dp b cn*) *D. melanogaster* males of the present study, both parental (wild and *al dp b cn* type) as well as three recombinant types (*al, cn* and *dp cn*) produced males and females in 1:1 ratio.

The MH-treated F_1 males produced a total of seven phenotypes in TC_1 progeny, comprising of two parental and five recombinant types. In three recombinant classles (*al b cn, al dp* and *b cn*), the number of males and females were not different statistically. However, in parental classes, number of wild type males were more than that of females ($p<0.005$) where as the number of *al dp b cn* type females were more than that of males ($p<0.001$). In recombinant type *cn*, number of female flies was more than that of male flies ($p<0.01$). Miglani and Mohindra (1986a) observed deviation in polled TC_1 progeny of untreated F_1 males from 1:1 sex-ratio for one parental (*dp b cn*) and one recombinant (*b cn*) type. Singh (1989) failed to observe in TC_1 progeny of control F_1 (+/*dp b cn*) males 1:1 sex-ratio for both the parental and two recombinant (*b cn* and *dp*) classes whereas in HAS-treated F_1 (+/*dp b cn*) males only one recombinant class (*dp b*) did not show 1:1 sex-ratio. In untreatd and HAS-treated F_1 (+/*al dp b*) males, it was noted that the males and females appeared in unequal frequency only in two recombinant cases *al* and *b*. In majority (87.33%) of the 71 classes studied by Preet(1989), the males and females were found to be in 1:1 ratio for all recombinant and parental classes in TC_1 progeny of control and EMS experiments.

5.5 Number of Recombinants Produced Per Male.

Different test cross progenies of MH experiment did not reveal same number of recombinants per F_1 male. In order to make a comparison, total number of male and female recombinants recovered in the pooled test cross progeny was divided by the total number of male and female recombinants recovered in the pooled test cross progeny was divided by the total number of males test crossed. In the present study, the number of recombinants produced per F_1 (+/*al dp b cn*) male treatd with MH was 3.65 and that in control experiment this value was 1.83. Singh (1992) observed 1.15 recombinants per F_1 (+/ *al dp b cn*) male treated with EMS. Miglani and Mohindra (1986a) reported 7.0 recombinants per EMS-treated F_1 (+/*dp b cn*) male. Number of recombinants recovered per F_1 (+/ *dp b cn*) male was 2.22 with 0.5% HAS wheras this value per F_1 (+/*al dp b*) male was 2.8 (Singh, 1989). With 0.25% and 2.5% formaldehyde, the number of recombinants per F_1 (+/*al dp b pr*) male were 1.0 and 3.12, respectively (Preet, 1989). Thus, the number of recombinants recovered in this study per 0.5% MH-treated F_1 male was lower than that recorded by Miglani and Mohindra (1986a) with 0.75% EMS, by Singh (1989) with 0.5% HAS, and by Preet (1989) with 2.5% formaldehyde. However, the number of recombinants observed per MH-treated F_1 males in the present study was higher than that reported per 0.25% formaldehyde-treated F_1 male by Preet (1989).

5.6 Reciprocal versus Non-Reciprocal Recombination

The number of complementary recombinants observed in the present study with 0.5% MH in *al-dp, dp-b* and *b-cn* regions when tested for 1:1 ratio revealed male recombination to the non-reciprocal in *dp-b* and *b-cn* regions and reciprocal in *al-dp* regions of *D. melanogaster* (Table 4.6). Miglani and Thapar (1983b), Miglani and Mohindra (1986a), Sharma and Swaminathan (1968) and Singh (1992) also reported induction of non-reciprocal recombination with 0.75% EMS in *dp-b* region. With belomyein also, non-reciprocal recombination was observed in this region (Demopoulous, 1980). Sobels and van Steenis (1957) observed nor-reciprocal male recombination with formaldehyde in *b-pr* region of *D. melanogaster* . Singh (1989) observed HAS-induced non-reciprocal recombination in *al-dp* and *dp-b* regions. Preet (1989) noted that formaldehyde-induced male recombination was of non-reciprocal type in *dp-b* and *b-pr* but not in *al-dp* region.

5.7 Confirmation of Recombinants

All the TC_1 recombinant males of *D. melanogaster* recoverd with 0.5% MH, in the present study, were further test crossed for phenotypic/genotypic confirmation. All of them produced the recombinant in addition to *al dp b cn* type flies in their TC_2 progenies and were thus verified. Schacht (1958) further bred 18 X-rays-induced cross overs of second chromosome markers of *D. melanogaster* and all of them were verified whem bred again.

Miglani and Mohindra (1986a), on the contrary, observed that not all but majority (84.9%) of 0.75% EMS-induced TC_1 recombinant males further test crossed could be verified. Miglani *et al.* (1990), reported verification of all te TC_1 recombinant males produced with 0.5% HAS. Preet (1989) also observed that all the recombinant males recovered with 0.25% and 2.5% formaldehyde were confirmed. Singh (1992) also reported verification of all the TC_1 recombinant males produced with 0.75% EMS.

In the TC_2 progeny of a TC_1 recombinant males, the recombinant and *al dp b cn* flies were expected to appear in 1:1 ratio. But this was not observed to be the case in all the TC_2 progenies. out of 87 (3 from control and 84 from MH experiment) TC_1 recombinant males verified (Table 4.7), in more than half (55.17%) of the TC_2 progenies in which recombinant phenotype ranged from 41.0 to 67.0 per cent, the two phenotypes statistically fitted in 1:1 Ratio while the ramining deviated significantly. The wide range of values in terms of per cent of recombinant type flies observed in TC_2 progenies of 87 TC_1 recombinant males test crosses showed a continuous variation (Fig.4.1) which suggested polygenic control on the expression of a TC_1 recombinant male in its TC_2 progeny.

5.8 Chromosomal Aberrations and Male Recombination

Woodruff and Thompson (1977) gave a hypothesis that male recombination events could occur by classical crossing-over, as in females, or by chromosome breakage and reunion events. There is some evidence, however, which suggests that male recombination does not occur by the same mechanism as female recombination, implying that male recombination is not occurring by classical crossing-over. Miglani and Thapar (1983a) suggested that high frequency of male recombination in *dp-b* region may be due to preferential chromosomal breakage by EMS. Miglani and Mohindra (1985) detected five inversions and two deletions in the TC_2 progeny larvae of TC_1 recombinant males produced with 0.75% EMS, three of these inversions overlapped the *dp-b-cn* region. From these observations these workers suggested role of chromosomal aberrations in male recombination with EMS, in *D. melanogaster*.

Efforts to understand the involvement of chromosomal aberrations in male recombination were continued by Singh (1989), using HAS as a probe, following the same approach as by Miglani and Mohindra (1985). Three inversions and one deletion were detected in TC_2 progeny larvae of three TC_1 recombinant males (Singh, 1989). Two inversions overlapped the *al-dp-b* chromosomal region studied. Singh (1992) found three deletions and two inversions in the TC_1 larvae, no chromosomal aberration was, however, detected after the TC_1 generation, in her study.

One of the objectives of the present study was to explore possible association of MH-inducued male recombination with chromosomal aberrations. In the present study, the F_1 (+/*al dp b cn*) larvae of *D. melanogaster* were found to posess three inversions, 2R(52A-52F), 2L(32F-36D) and X(5A-6F). The TC_1 larvae were also found to posses three inversions, 2L(22D-26A), 2L(21E-23F) and 3L(69F-71A) in the salivary chromosomes. Two deletions, 2L(34D-38D) and 2L(32A-34F), and four inversions, 2L(23F-26A), 2L(24A-26B), 3L(67E-69D) and 2L(24A-26B), were detected in the TC_2 larvae that had descended from different TC_1 recombinant males. Two inversions, 2L(36D-38F) and 2L(24A-26B), were observed in FS_1 larvae that had descended from TC_2 recombinant males. Only one inversion, 3L(67D-69F), was observed in FS_2 larvae that had descended from a TC_1 recombinant male. The chromosomes of larvae sampled in the present study during different generations are mapped on cytogenetic map prepared by King (1975). A study of this map shows that out of a total 15 chromosomal aberrations detected in the larvae sampled from different generations, in present study, 10 lie in left arm of chromosome 2 and 8 of them overlap the *dp-b* region. Incidently, this is the region in which highest frequency of male recombination was observed in the present study.

No chromosomal aberration was detected from heterozygotes of homozygous male recombinant lines (HMRLs) constructed from two spontaneous and five HAS-induced male recombinants of Singh (1989) and five spontaneously and eighteen formaldehyde-induced male recombinants of Preet (1989). One inversion, 2L(31A-34C), observed with 0.25% formaldehyde in F_1 (+/*al dp b pr*) larvae by Preet (1989) may be involved in induction of male recombination as it was included in *dp-b*, the region in which highest frequency of male r ecombination was observed by her. No inversion was, however, observed in the progeny larvae of HMRLs, in the her study.

Out of a total of 15 aberrations detected in larvae sampled from different generations, in the present study, 8 overlapped the *dp-b* region where maximum frequency of recombinants was also obtained. Are any of these 8 aberrations involved in induction of male recombination ? One inversion, 2L(24A-26B), detected in TC_2 larvae was also observed in an FS_1 larva; both these TC_2 and FS_1 larvae had descended from one and the same TC_1 recombinant male MH74.82 (Table 4.10). This inversion partly falls in *dp-b* region. May be this inversion is involved in induction of male recombination with MH. We certainly have, at present, no evidence to prove this point. We also do not have any reason to rule out involvement of this or any of other chromosomal aberrations reported in this study because intrachromosomal effects on induction of male recombination are known (Singh and Singh, 1988).

If the hypothesis about the role of chromosome breakage and reunion in male recombination, as suggested by

Woodruff and Thompson (1977), is vaild, our belief is that the breaks responsible for male recombination in *D. melanogaster* may not finally lead to the formation of chromosomal aberrations as such. If at all the chromosomal aberrations play role in induction of male recombination, they must be having some indirect role in this process. Since chromosomal aberrations were observed in F_1 larvea, the time at which male recombination was probably induced, but the same were not recovered in progenies of homozygous male recombinants, it may be suggested that such chromosomal aberrations as are involved in induction of male recombination must be of unstable nature or of a very small magnitude which may not be detectable at the magnification (1,000X) studied.

The role of unstable chromosomal aberrations in male recombination has also been suggested Yannopoulous *et al.* (1982; 1983) fopr male recombination factor 31.1MRF and for chemically-induced male recombination (Miglani *et al.*, 1990; Miglani and Preet, 1990). Regarding the mechanism through which EMS may have induced male recombination and chromosomal aberrations in *D. melanogaster*, in the present study, one may speculate that since EMS is known to produce mutation through alkylation it may have induced chromosomal aberrations as well as male recombination through the same process.

5.9. Male Recombination Potantial in HMRLs

A few attempts have been made in the last two decades to study male recombination and other related phenomenon in lines constructed from *Drosophila* flies captured from natural populations. Spontaneous male recombination and visible mutations were observed in *st-cu*, *st-P^p* and *b-cn* regions of second chromosome derived from an isogenic line, ID (Kidwell and Kidwell, 1975b). Woodruff and Thompson (1977) observed that all recombinants males bred true as second chromosome recombinants in isolated recombinant lines. They also observed equal recovery of reciprocal exchanges in the *b-cn* and *cn-bw* regions but not in *dp-b* region.

In the present study, success was achieved in construction of homozygons male recombinant lines (HMRLs) from the 2 spontaneous and 50 EMS-induced TC_1 male recombinants of *D. melanogaster*. Potential of only 25 HMRLs produced from MH-induced TC_1 recombinant males could be studied, in reciprocal crosses, for producing male recombination as all the HMRLs could not be maintained in the laboratory with equal facility. For studying male recombination potential of HMRLs, a male /female from an HMRLs were crossed to female/male from a complementary stock and the F_1 males thus obtained were crossed with *al dp b cn* females. The ability of *D. melanogaster* HMRLs constructed from chemically-induced recombinants males to produce male recombination was studied, following this approach by Miglani and Singh (1992b).

Out of 50 test corss progenies screened in the present investigation, 41 (82.0%) revealed male recombination. Male recombination observed in different test cross progenies ranged from 3.16-29.50 per cent when the F_1 males were produced using HMRLs as female parent and from 2.19-21.59 per cent when the F_1 males were produced using HMRLs as a male parent. The overall range of per cent male recombination in different test cross progenies in all the reciprocal crosses combined was 2.19 to 29.50.

Thus the majority of the HMRLs of *D. melanogaster* constructed from MH-induced TC_1 recombinant males possesed inherent pootential for producing male recombination. In the expriments conducted by Miglani and Singh (1992b) 34 HMRLs constructed from EMS-induced TC_1 recombinant males were studied in reciprocal crosses to examine their potential for producing male recombination. Out of 68 test cross progenies studied by them only 13 revealed male recombination. Male recombination recorded in different TC_1 progenies ranged from 3.10-7.21 per cent when the F_1 males were produced using HMRLS as a female parent and from 6.31-18.07 per cent when F_1 males were produced using HMRL as male parent. The overall range of per cent male recombination in different test cross progenies in all the reciprocal crosses combined was 3.10-18.07.

The HMRLs obtained in the present study with HM exhibited broader range than these conducted by Singh (1992) with EMS in terms of per cent male recombination in *D. melanogaster*.

5.10 Conclusions

Induction of male recombination with maleic hydrazide (MH) has been reported here for the first time in

Drosophla. MH has been found to be a potent chromosome breaking agent and recombinogen. A total of 15 chromosomal aberrations were detected in F_1, TC_1, TC_2, FS_1 and FS_2 generations out of which 10 were located in chromosome 2L and 8 of them overlapped the *dp-b* region. Incidenty, *dp-b* is the region of chromosome 2 of *D. melanogaster* in which highest frequency of male recombination has been noted earlier as well as in the present study.

Do any of these chromosomal aberrations play role in induction of male recombination with MH in *D. melanogaster*? is an obvious question. To answer this, homozygous male recombinant lines (HMRLs) were constructed from MH-induced TC_1 recombinant males. Males/females from HMRLS when crossed with females/ males form an aberration-free complementary stock did not reveal and chromosomal aberration in the resultant F_1 larvae. Although no direct association was seen of a particular stable chromosomal aberration with a recombinant phenotype, the role of chromosomal aberrations in male recombination in *D. melanogaster* is suggestive. The involvement of chromosome breaks/aberrations in male recombination in *D. melanogaster* thus still remains an answered question and warrants further investigation.

CHAPTER VI

SUMMARY

Malic hydrazide (MH) (0.5%) was mixed to the standard food medium in ratio of 1:9 and fed to the developing F_1 individuals of genotype +/*aristaless dumpy, black cinnabar* (*al dp b cn*) during the entir larval life of *Drosophila melanogaster* to induce male recombination and chromosomal aberrations and to find out if these chromosomal aberrations descended through several generations (F_1, TC_1, TC_2, FS_1, FS_2 and FS_3) till a homozygous male recombinant line (HMRL) was constructed. The salivary chromosomes of the progeny larvae of the HMRLs were also examined for the presence of chrosomal aberrations and to find out if there is any association of chromosomal aberrations with male recombination. The inherent ability of HMRLs to produce male recombination was also studied. All the experiments were done at 25 ± 1°C. Control experiments were also run simultaneously. The salient findings are summerized below:

1. Sixty untreated F_1 males test crossed, produced a pooled TC_1 progeny of 8001 flies inculding 11 recombinants of three phenotypes (*al*, *dp cn* and *cn*). Out of 90 MH-treated F_1 males test crossed, a pooled TC_1 progeny of 11843 flies with 211 recombinants of five phenotypes (*al b cn*, *al dp*, *b cn*, *dp* and *cn*) was obtained where *b cn*

was the most and *al dp* the next most frequent recombinant observed.

2. In control experiment, in 6 out of 60 TC_1 progenies (each produced by a different F_1 male) in which recombinants were recovered, frequency of recombinants ranged from 0.85 to 1.89 per cent. In MH experiment, in 23 out of 90 TC_1 progenies in which recombinants were observed, frequency of recombinants ranged from 2.325 to 15.85 per cent. The overall per cent male recombination observed with MH was the highest in *dp-b* (1.661) followed by *al-dp* (0.372) and *b-cn* (0.011) regions.
3. The segregation patterns in TC_1 progenies of control and MH experiment were non-Mendelian at all the four loci studied, with frequency of "wild type" allels being higher over their respective "mutant" alleles.
4. The number of TC_1 males and females recovered in the two parental (wild and *al dp b cn* type) and three recombinant classes (*dp cn, al* and *cn*) of control experiment were in 1:1 ratio. In MH experiment, number of TC_1 males and females produced in both the parental classes (wild and *al dp b cn* type) did not appear in 1:1 ratio. Among the recombinant classes, the number of males and females noted in *b cn, al dp, al b cn* and *dp* recombinants in TC_1 progeny were same but in *cn* recombinant type, the two sexes did not appear in 1:1 ratio, statistically.
5. The complementary classes observed in TC_1 progenies, when tested for 1:1 raito revealed that wild type phenotype

was predominant over *al dp b cn* in control as well as MH experiment. In control, the two classes comprising each of the three pairs of complementary recombinant classes (*al vs dp b cn ; al dp b vs cn* and *al b vs dp cn*) were observed in equal frequency. In MH experiment, two classes of each of the two complementary pairs of recombinant phenotypes, *al dp* vs *b cn* and *al dp b* vs *cn*, showed the number of flies of latter pheontypes to be in majority over the former. For the complementary pair *al b cn* vs *dp*, number of flies in the two classes were in equal frquency, statistically.

6. Out of 11 and 211 TC_1 recombinants observed in control and MH expriments, 3 and 84, respectively were males; all these recombinant males were verified genotypicaly/phenotypically by further test crossing. The frequency of recombinant type flies in 84 TC_2 progenies of TC_1 recombinant male of MH experiment ranged from 5.50 to 84.37 per cent. A total of 50 HMRLs (33 *b cn*, 8 *al dp*, 4 *al b cn* and 5 *dp*) were constructed through several full-sib matings of phenotypically idenitcal TC_2 recombinant males and females produced by a particular MH-induced TC_1 recombinant male.
7. Salivary chromosomes were studied, at magnification 1,000X. Only those experiments in which chromosomal aberrations were detected are described. In F_1 gerneration, three inversions, 2R(50A-52F), 2L(32F-36D) and X(5A-6F), were detected in the MH experiment out of 3999 cells screened. In TC_1 generation, in MH experiment,

15,000 salivary cells were scored wherein 2 inversions, 2L(22D-26A) and 2L(21E-23F), were detected in different cells of the same larva. Another inversion, 3L(69F-71A), was observed in cells of a different TC_1 larva. In TC_2 generation, from 24757 salivary cells screened two deletions, 2L(34D-38D) and 2L(32A-34F) and four inversions, 2L(23F-26A), 2L(24A-26B), 3L(67E-69D) and 2L(24A-26B), were observed in MH experiment. In FS_1 generation of the MH experiment, 10,040 cells were screened and 2 inversions, 2L(36D-38F) and 2L(24A-26B), were observed. In 13,567 cells examined in FS_2 generation of MH experiment, only one inversion, 3L(67D-69F), was observed. No aberration was observed in salivary cells of FS_3 and those of F_1 generation produced by crossing HMRLs x complementary phenotype, or its reciprocal. In this study a total of 15 chromosomal aberrations were detected, ten of them were located in chromosome 2L and eight of them overlapped *dp-b*, the region in which highest frequency of male recombination was obseved with MH.

8. To find out if the HMRLs constructed possesed inherent ability to produce male recombination, the F_1 males obtained from the crosses HMRL x complementary phenotype, or its reciprocal, were test crossed with *al dp b cn* females to obtain test cross progeny. It was possible to conduct male recombination studies on only 25 (16 *b cn*, 4 *al dp*, 3 *dp* and 2 *al b cn*) HMRLs, in reciprocal crosses. Male recombination was produced in 41 out of 50

test crosses. The overall per cent male recombinants observed in TC_1 progenies when F_1 male was produced using HMRLs as female parent ranged from 3.16 to 29.50 wheras when F_1 male was produced using HMRLs as male parent, this value ranged from 2.19 to 21.59. In the present study, out of 25 HMRLs examined 23 posessed inherent ability in one or both of the reciporcal crosses to produce male recombination in *D. melanogaster*.

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