GENETIC ANALYSIS OF THE PROXIMAL HETEROCHROMATIN OF CHROMOSOME-2 OF DROSOPHILA MELANOGASTER

by

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In the Department of Zoology

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

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ABSTRACT

The genetic function of Drosophila heterochromatin has been debated since its earliest description by Heitz (1933). To examine the genetic composition of the proximal region of chromosome 2 of Drosophila melanogaster, the generation of proximal deficiencies by the detachment of compound second autosomes appeared to be a promising method. Compound second autosomes were detached by gamma radiation. A fraction of the detachment products were recessive lethals owing to proximal deficiencies. Analysis of these detachment products by inter se complementation, pseudo-dominance tests with proximal mutations and alleleism tests with known deficiencies, provided evidence for at least two loci between the centromere and the light locus in 2L and one locus in 2R between the rolled locus and the centromere. These data in conjunction with cytological observations further demonstrate that rolled and light are located within the proximal heterochromatin of the second chromosome. To further this analysis, lethal alleles of the largest 2L and 2R proximal deficiencies were generated, employing, as a mutagen, ethyl methane sulphonate (EMS). Analysis of the 118 EMS induced recessive lethals and visible mutations recovered provided evidence for seven loci in the 2L heterochromatin and six loci in the 2R heterochromatin, with multiple alleles being obtained for most sites. Of these loci, one in 2L and two in 2R fall near the heterochromatic-euchromatic junction of 2L and 2R respectively. None of the 113 EMS lethals behaved as a deficiency, thereby confirming that, in Drosophila, the EMS mutagenesis method of Lewis and Bacher (1968) results in true "point" mutations. All of the heterochromatic loci uncovered in this study appear to be non-repetitive cistrons. Thus functional genetic loci are found in heterochromatin, albeit at very low density relative to euchromatin.
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CHAPTER I

GENERAL INTRODUCTION
The biological function of heterochromatin has been debated since its first clear description by Heitz (1928, 1929). This debate became one of broad interest with the demonstration of the ubiquity of heterochromatin among plants and animals (Brown 1966).

Nineteenth century cytologists, in dissecting the mitotic cycle, demonstrated that after nuclear division chromosomes unravelled (decondensed) during the formation of the interphase nucleus. Thus most of the chromosomal material was condensed only for part of the cell cycle, namely prophase through anaphase, and in telophase began to decondense. At interphase the chromosomes could not be resolved. However, in these interphase nuclei, bodies of dark staining material were often observed. Heitz demonstrated that these darkly staining bodies corresponded to chromatin which remained condensed throughout the cell cycle. This permanently condensed chromatin Heitz termed "heterochromatin". In interphase nuclei the heterochromatic blocks were referred to as "chromocenters". Often chromocenters fused to form one or several large composite chromocenters. Since Heitz was of the opinion that the chromosomal genes could be active only in noncondensed chromatin, he suggested that heterochromatic regions were genetically inert.

A number of cytological properties are diagnostic of heterochromatin. In most animal species heterochromatin is pericentromeric, while in plants terminal heterochromatin is common. Further, throughout prophase heterochromatic regions are considerably denser than the remaining euchromatin. Homologous chromosomes are homologous in their sites of heterochromatin, which remain constant. Finally, hetero-
chromatic regions exhibit the property of chromatid apposition during mitotic prophase.

The heterochromatin described above is "classical" or "constitutive" heterochromatin (Brown 1966). A second type of heterochromatin termed "facultative" is that associated with chromosomal inactivation through condensation of one of a pair of homologous chromosomes in the soma or of a univalent in one cell type as, for example, the condensation of the X-chromosome during spermatogenesis in some insects. Throughout the following text the term heterochromatin is used strictly in reference to constitutive heterochromatin.

From their cytogenetic studies on *Drosophila melanogaster* Heitz (1933) and Kaufmann (1934) facilitated inference as to the genetic constitution of heterochromatin. They found that the entire Y-chromosome, the proximal 1/3 to 1/2 of the X-chromosome, and the proximal 1/4 of each of the arms of the metacentric major autosomes, chromosomes 2 and 3, were heterochromatic at prometaphase. With respect to the Y-chromosome these observations were of considerable significance as the earlier studies of Bridges (1916) had demonstrated an apparent lack of genes in the Y-chromosome. Bridges and his colleagues discovered no instance of a Y-linked gene, and, further, Bridges found that nullo-Y males (XO) were of perfectly normal phenotype, viability and behaviour, but sterile. Thus it appeared that the completely heterochromatic Y-chromosome, although larger than the X-chromosome, for which many genetic loci were known, contained no genes.

However, the description of the Y-chromosome as completely heterochromatic and genetically inert was to be modified. Several secondary
constrictions were described in the Y-chromosome (reviewed in Cooper 1959). Further, as Bridges' (1916) discovery of the sterility of XO males anticipated, specific fertility factors were subsequently revealed (reviewed in Brosseau 1960 and Hess and Meyer 1968). The numbers of genetic sites (nucleolus organizer plus fertility factors equalling 8) and secondary constrictions (7-9) are in close agreement, suggesting that the secondary constrictions correspond to Y-chromosome genetic loci. Hess and Meyer's (1968) findings in Drosophila hydei spermatogonia of lampbrush chromosome loops at the sites of Y-chromosome secondary constrictions strengthens this hypothesis. Thus, although the heterochromatic Y-chromosome does contain genetic loci, these loci would appear to reside at the sites of the secondary constrictions and, therefore, are not truly heterochromatic.

Similarly, Heitz's and Kaufmann's (loc. cit.) cytological findings were, in the light of Heitz's speculation of the genetic inertness of heterochromatin, in agreement with the work of Muller and Painter (1932) on gene localization in the X-chromosome of Drosophila melanogaster. Muller and Painter demonstrated that few if any gene loci were located in the proximal third of the X-chromosome, precisely that region which Heitz and Kaufmann found to be heterochromatic. The recent detailed fine structure analysis of the X-chromosome proximal region by Schalet and Lefevre (1973) demonstrates that only one locus, bobbed (bb) (which was previously known to be in heterochromatin), is definitely in the X-chromosome heterochromatin. The bobbed locus has been identified as the nucleolar organizer of the X-chromosome (Ritossa, Atwood and Spiegelman 1966) and is associated with a prominent secondary constriction.
Secondary constrictions, in addition to the nucleolar constriction, have been reported for the X-chromosome proximal heterochromatin (Cooper 1959) and these may correspond to further loci, which are as of yet undefined.

Little is known of the genetic constitution of the heterochromatic segments of the major autosomes of Drosophila, although it has been speculated that light (Schultz 1936) and rolled (Morgan, Schultz and Curry 1940) are in the proximal heterochromatin of chromosome-2.

In addition to permanent heteropycnosis, there are other long established differences between heterochromatin and euchromatin in Drosophila. Little or no recombination occurs in Drosophila heterochromatin (Baker 1958). Further, cytophotometric data (Rudkin 1969) confirms the earlier conclusion of Heitz (1933) that heterochromatin does not replicate during polytene chromosome formation. Drosophila heterochromatin initiates DNA replication much later in the S phase of the cell cycle than euchromatin (Barigozzi et al. 1966). In fact, DNA synthesis late in the S phase of the cell cycle is characteristic of heterochromatin in all species (Lima-de-Faria and Jaworska 1968). Modern biochemical and cytochemical studies have demonstrated that, as for most animal species, Drosophila heterochromatin is enriched in highly repetitive DNA (Botchan et al. 1971; Gall et al. 1971; Peacock et al. 1973). In most animal species heterochromatin is enriched in highly repetitive DNA; the Chinese hamster (Cricetulus griseus) is the only known exception (Comings and Mattoccia 1972; Arrighi et al. 1974). The repetitive sequences are often very short in length, of the order of magnitude of 10 nucleotides, and thus in all probability would not
be transcribed (Yunis and Yasmineh 1971). In *Drosophila melanogaster* there are seven highly repetitive DNA sequences which make up the bulk of the constitutive heterochromatin of this species (Peacock et al. 1973; Sederoff et al. 1975), one of these sequences being an AGAAG pentamer, which is localized to the heterochromatin of the second and Y chromosomes.

In assigning functions to heterochromatin, biologists have been highly imaginative. The phenomenon of position-effect variegation (reviewed in Lewis 1950; Hannah 1951; Baker 1968) leads many to postulate the existence, within heterochromatin, of regulatory genes which affect neighbouring loci. However, the mechanism of position-effect variegation remains unclear. Others have postulated that heterochromatin plays a role in chromosome pairing and segregation during meiosis (reviewed in Yunis and Yasmineh 1971), or that pericentromeric heterochromatin protects the centromere during nuclear division (Walker 1971). While these and many other functions have been proposed (reviewed in Hannah 1951; Cooper 1959; Yunis and Yasmineh 1971), there is little, if any, evidence to support any of the postulated functions.

While the experimental findings summarized above appear to demonstrate that heterochromatin is genetically inert, a conclusion that is today receiving wide acceptance, the evidence remains consistent with an hypothesis of a very low gene density in heterochromatin relative to euchromatin, as no single heterochromatic chromosome region has been subjected to a truly thorough genetic and cytogenetic analysis. The obvious choice of organism in which to attempt such a complete genetic analysis of a heterochromatic region is *Drosophila melanogaster*, whose
genetics and, in a qualified sense, cytogenetics are more complete than any other higher organism's.

In a thorough analysis of a heterochromatic chromosome segment there are a number of things one would want to accomplish. First, one would want to demonstrate the number and physical location of genes associated with that heterochromatic segment. One would want to know whether these genes are actually in or merely adjacent to heterochromatin (i.e. at the heterochromatic-euchromatic junction) and if there is any association of these genes with secondary constrictions. In addition, one would want to investigate the nature of these loci in terms of whether they are repetitive or nonrepetitive sites and of their involvement in development. Further, one would want to investigate the effect of deletions and duplications for all or part of this heterochromatic segment upon meiosis, for it has been suggested that heterochromatin may bring homologous chromosomes together during meiosis and that centromeric heterochromatin may protect the centromere from breakage during the course of the meiotic division; thus predicting that heterozygosity for partial or complete deficiencies of a centromeric heterochromatic segment may result in elevated rates of nondisjunction or chromosome loss. Finally, the thorough genetic analysis of such a heterochromatic chromosome segment should provide the material to investigate definitively whether or not recombination occurs in heterochromatin.

The heterochromatic region I have chosen to analyze is the proximal region of chromosome-2 of *Drosophila melanogaster*.
CHAPTER II

ANALYSIS OF DETACHMENT PRODUCTS
OF COMPOUND SECOND AUTOSOMES
INTRODUCTION

As an initial approach towards examining the genetic composition of the proximal heterochromatin of chromosome-2 in *Drosophila melanogaster*, the detachment of compound autosomes appeared a promising method for generating proximal deficiencies. It has been well documented that detachments of compound-X chromosomes are generated by interchanges between heterologous chromosomes (Parker 1954; Abrahamson, Herskowitz and Muller 1956; Parker and Hammond 1958; Parker and Williamson 1970). Baldwin and Suzuki (1971), upon finding proximally located recessive lethals associated with standard thirds recovered from radiation treated compound-3-bearing females, proposed that radiation-induced detachments of compound autosomes provided a means of generating autosomal proximal deficiencies. I was cautioned, however, by the fact that Parker (1954) noted a considerable variation in lethal frequencies associated with detachments from various attached-X stocks, suggesting that some compound-X chromosomes had accumulated spontaneous lethals. He commented that "these spontaneous lethals probably accumulate near the centromere where homozygosis frequency is low and selection relatively ineffective". Nevertheless, I found that through using homogeneous populations of compound autosomes, proximal deficiencies could be identified as products of compound-2L and compound-2R detachments. Moreover, by using the deficiencies so generated, a number of genetic loci have been localized to the centric heterochromatin of chromosome-2.
MATERIALS AND METHODS

Mutations and chromosome rearrangements: A brief description of the mutations used in this study is provided in Table I. The two multiple-break inversions employed as balancers for the reconstituted chromosomes were 1) \(\text{In}(2L)\text{bw}^{VI}\); an inverted second chromosome identified by a dominant brown-variegated \((\text{bw}^{VI})\) eye phenotype and 2) \(\text{In}(2L)\text{SM1,Cy}\); an effective balancer for the entire length of chromosome-2, marked by a dominant curly wing \((\text{Cy})\) phenotype. Further details on the above mutants and rearrangements are given by Lindsley and Grell (1968).

Compound autosomes: Compound-2 strains used include:

1) \(\text{C}(2L)\text{SH3,}^{+};\text{C}(2R)\text{SH3,}^{+}\), 2) \(\text{C}(2L)\text{SH1,}^{+};\text{C}(2R)\text{SH3,}^{+}\), 3) \(\text{C}(2L)\text{SH3,}^{+}\); \(\text{C}(2R)\text{VK1,bw}\), 4) \(\text{C}(2L)\text{VY1,b pr};\text{C}(2R)\text{P1,px}\). Each newly generated \(\text{C}(2L)\) or \(\text{C}(2R)\) chromosome is assigned an alphanumeric code and established in an independent line. The first code letter identifies the place of origin (i.e. \(P = \text{Pasadena}; S = \text{Storrs}; V = \text{Vancouver}\)). Pertinent genetic properties of the compound autosomes used in this study are described under related headings in the RESULTS AND DISCUSSION section of this chapter.

Recovery of compound-2 detachment products: Compound-2 virgin females were treated with 0 (controls) or 2000 rads of gamma radiation from a \(^{60}\text{Co}\) source and crossed to heterozygous \(\text{In}(2L)\text{bw}^{VI}/\text{In}(2L)\text{SM1,Cy}\) males. Rare surviving progeny either with a brown variegated eye \((\text{bw}^{VI})\) or with a curly wing \((\text{Cy})\) phenotype were scored as putative detachment products (i.e. reconstituted standard second chromosomes) and individually mated to \(\text{In}(2L)\text{bw}^{VI}/\text{In}(2L)\text{SM1,Cy}\) flies of the opposite sex. From each line crosses were made between F2 virgin females and males, each heterozygous
TABLE I

Recessive lethal and visible mutations used as genetic markers on compound autosomes and for complementation analysis.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Map position</th>
<th>Chromosome arm</th>
<th>Phenotype</th>
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<tbody>
<tr>
<td>b</td>
<td>2-48.5</td>
<td>2L</td>
<td>black body</td>
</tr>
<tr>
<td>pr</td>
<td>2-54.5</td>
<td>2L</td>
<td>purple eyes</td>
</tr>
<tr>
<td>Bl</td>
<td>2-54.8</td>
<td>2L</td>
<td>short bristles, homozygous lethal</td>
</tr>
<tr>
<td>esc</td>
<td>2-54.9</td>
<td>2L</td>
<td>extra sex combs on male</td>
</tr>
<tr>
<td>t(2)arc</td>
<td>2-55</td>
<td>2L</td>
<td>homozygous lethal</td>
</tr>
<tr>
<td>tri</td>
<td>2-55</td>
<td>2L</td>
<td>dark streak on thorax</td>
</tr>
<tr>
<td>lt</td>
<td>2-55.0</td>
<td>2L</td>
<td>yellowish-pink eyes</td>
</tr>
<tr>
<td>rl</td>
<td>2-55.1</td>
<td>2R</td>
<td>rolled wing edges</td>
</tr>
<tr>
<td>M(2)S10</td>
<td>2-55.1</td>
<td>2R</td>
<td>thin bristled Minute, homozygous lethal, deficient for rl</td>
</tr>
<tr>
<td>M(2)S8</td>
<td>2-55.1</td>
<td>2R</td>
<td>Minute, homozygous lethal and with M(2)S10 and M(2)S4, deficient for stw.</td>
</tr>
<tr>
<td>M(2)S4</td>
<td>2.55.1-.2</td>
<td>2R</td>
<td>Minute, homozygous lethal and with M(2)S10 deficient for stw and ap</td>
</tr>
<tr>
<td>stw</td>
<td>2-55.1</td>
<td>2R</td>
<td>yellow hairs, pale bristle tips</td>
</tr>
<tr>
<td>ap</td>
<td>2-55.2</td>
<td>2R</td>
<td>wings missing</td>
</tr>
<tr>
<td>msf</td>
<td>2-55.2</td>
<td>2R</td>
<td>wings short, crumpled, legs short, eyes misformed</td>
</tr>
<tr>
<td>pk</td>
<td>2-55.2</td>
<td>2R</td>
<td>anteriorly slanted costal hairs</td>
</tr>
<tr>
<td>tuf</td>
<td>2-55.5</td>
<td>2R</td>
<td>tuft of hairs between antennae and eyes</td>
</tr>
<tr>
<td>ltd</td>
<td>2-56</td>
<td>2R</td>
<td>yellowish-pink eyes</td>
</tr>
<tr>
<td>cn</td>
<td>2-57.5</td>
<td>2R</td>
<td>bright red eyes, colorless ocelli</td>
</tr>
<tr>
<td>bw</td>
<td>2-104.5</td>
<td>2R</td>
<td>brown eyes</td>
</tr>
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for the putative detachment and \text{In(2LR)SM1,Cy}. Lethal and semi-lethal detachments were identified in the F3 progeny and maintained as balanced heterozygotes over \text{In(2LR)SM1,Cy}. Following careful examination of their phenotype, fully viable homozygous detachments were discarded.

**Complementation tests on lethal detachments:** All lethal detachments recovered from \text{C(2L)SH3,+/C(2R)SH3,+} and \text{C(2L)SH3,+/C(2R)VKt,bw} treated females (Experiments 1 and 2 respectively under Results) were tested for complementation in all possible inter se combinations and against the proximal mutations and deficiencies listed in Table 1. (Lethal detachments were also tested against the recessive visible roughish, \text{rh}. However, the reported location of \text{rh} at the base of 2L proved to be erroneous. For details see Appendix IV.) In addition, all lethal-bearing detachments were tested genetically for \text{X;2, 2;3} and \text{2;4} translocations. Each complementation test involved 2 or 3 pairs of parents brooded for four days in shell vials containing standard Drosophila medium. Reciprocal crosses were made in a number of tests. Progeny were screened for 20 days following the first observed eclosions. Since all recessive lethal detachments were balanced over \text{In(2LR)SM1}, which carried the dominant Curly (\text{Cy}) wing marker, the criterion for complementation was the recovery of straight (normal) winged progeny. All experiments were performed at 25\degree C.

**Cytological analysis:** Where described, polytene chromosomes from salivary glands and mitotic chromosomes from larval ganglia were prepared by aceto-lacto-orcein squash techniques (Appendices II and III). Photomicrographs were prepared using a Zeiss photomicroscope equipped
with phase contrast optics.

Origin of compound second autosome detachments: The data in Tables 2 and 3 are from a series of initial experiments conducted to examine the source and expected recovery of reconstituted, standard second chromosomes from compound-2 female parents. Notable recoveries of reconstituted autosomes have been obtained as apparent induced detachment products from gamma- or X-ray treated compound-2 (Bateman 1968, Hilliker 1972) and compound-3 (Chovnick et al. 1970, Baldwin and Suzuki 1971) females. However, I viewed these tests as essential to my analysis of detachment products since although spontaneous compound-autosome revertants have been reported (Leigh and Sobels 1970), their frequency of formation is unknown.

For these tests I used $C(2L)\text{VY, b pr; }C(2R)\text{P, px}$ virgin females aged 72 ± 24 hours. They were divided into four groups, the first group (the control) received 0 rads, two groups received 2000 rads of gamma radiation, and the fourth group, 4500 rads. For the control and two treatment groups (2000 and 4500 rads), single females were placed in vials with two $\text{In(2LR)SM1, Cy/In(2LR)bw V1}$ males. For the remaining treatment group (2000 rads), two females were placed in each mating vial. Since only products of exceptional meiotic events will be obtained from such a cross, an estimated relative productivity of these females was gained through mating approximately 10 percent of the females from each (treatment and control) group to their compound-2 brothers. After seven days of egg laying, parents were discarded. F1 progeny were counted and classified, and where necessary, F1 females were tested for triploidy. Vials not
yielding progeny were examined for the presence of fertilized eggs.

The estimated frequencies of detachment were based on the following argument. Segregation of C(2L) and C(2R) during meiosis in females approaches 100 percent in the absence of heterologous rearrangements, whereas in males, as inferred from genetic and egg hatch studies, compound-autosome assortment is approximately random (Holm et al. 1968, Grell 1970, Holm and Chovnick 1975). Consequently, only one quarter of the fertilized eggs in the multiplier vials (i.e. the compound by compound crosses) are expected to produce viable progeny. In contrast, when compound-bearing females are crossed to standard males, an egg pronucleus containing a reconstituted second chromosome will have, in theory, a 100 percent chance of recovery. The estimated frequencies of recoverable detachments, therefore, were calculated by dividing the number of putative reversions by the product of four times the mean number of progeny per multiplier vial times the number of experimental vials containing fertilized eggs. These results are presented (in Table II) primarily to provide a relative comparison between the frequencies of spontaneous and radiation induced events.

It is evident that the vast majority of reconstituted autosomes recovered arose as products of radiation induced detachments. In addition, as shown in Table III the recovery of reversion products followed a Poisson distribution in the radiation experiments, thereby implying that, for the most part, each compound-compound interchange product arose independently during meiosis.
## TABLE II

Estimated frequency of putative reconstituted standard second chromosomes recovered as detachment products from compound-2 females.

<table>
<thead>
<tr>
<th>Treatment in radios</th>
<th>Number of progeny per multiplier vial ± S.E.</th>
<th>Number of vials containing fertilized eggs</th>
<th>Estimated total number of zygotes</th>
<th>Number detachment products recovered</th>
<th>Estimated frequency of detachments in percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>27.43±1.12</td>
<td>1683</td>
<td>185,000</td>
<td>4</td>
<td>0.002</td>
</tr>
<tr>
<td>2000</td>
<td>23.47±1.36</td>
<td>1104</td>
<td>104,000</td>
<td>179</td>
<td>0.17</td>
</tr>
<tr>
<td>2000*</td>
<td>32.30±0.53</td>
<td>935</td>
<td>121,000</td>
<td>259</td>
<td>0.21</td>
</tr>
<tr>
<td>4500</td>
<td>23.16±1.22</td>
<td>1392</td>
<td>129,000</td>
<td>332</td>
<td>0.26</td>
</tr>
</tbody>
</table>

*Two female parents per mating vial
### TABLE III

Distribution of putative reconstituted standard seconds from compound-2 females treated with gamma-radiation.

<table>
<thead>
<tr>
<th>Dose (rads)</th>
<th>Female parents per vial</th>
<th>Number of vials</th>
<th>Progeny per vial</th>
<th>Goodness of fit $(\chi^2)^*$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>1</td>
<td>Expected</td>
<td>938.89</td>
<td>152.23 12.34 0.68</td>
<td>0.0112 .9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Observed</td>
<td>940</td>
<td>151  11  2</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>2</td>
<td>Expected</td>
<td>708.87</td>
<td>196.36 27.20 2.51</td>
<td>0.1239 .5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Observed</td>
<td>708</td>
<td>196  30  1</td>
<td></td>
</tr>
<tr>
<td>4500</td>
<td>1</td>
<td>Expected</td>
<td>1086.9</td>
<td>259.23 30.91 2.458</td>
<td>1.9591 .1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Observed</td>
<td>1103</td>
<td>249  37  3</td>
<td></td>
</tr>
</tbody>
</table>

* Classes 2 and 3 were pooled for the $\chi^2$ test (df=1).
RESULTS AND DISCUSSION

An interesting parallel can be drawn between formation and detachment of compound autosomes. Studies on the detachment of compound-X chromosomes disclose that interchange between heterologous chromatids is responsible for the separation of attached arms (Parker 1969). The interchange is, in essence, a translocation event followed by the segregation of reciprocal products; as a consequence, thereof, one half of the translocation product is recovered. While, classically, compound chromosomes, especially in plants, were viewed as isochromosomes arising from centromeric misdivision (for example see Darlington 1939, 1940) - a view acknowledged by Bateman (1968) as a possible source of compound autosome formation in Drosophila - we have since discovered that many newly generated compound-2 chromosomes carry duplications for the most proximally known markers on the opposite arm (see Appendix IV). From this we infer that several compound autosomes are also heterozygous for proximal deficiencies. Taken together, the above observations support the notion that both the formation and the detachment of compound chromosomes arise from interchanges between heterologous chromatids and provide the frame of reference for the models offered in explaining the results that follow.

In separate experiments, virgin females from three different compound-2 lines were treated with 2000 rads of $^{60}$Co radiation and mated with In(2LR)bw$^{vl}$/In(2LR)SM1,Cy males. Each putative detachment product recovered in a fertile individual was tested for homozygous lethality (see Materials and Methods for details). The results of these tests,
presented in Table IV, show a wide range in the proportion of lethal-bearing detachments recovered from the three separate lines. These differences (from approximately .25% in Experiment 2 to almost 100% in Experiment 3) will derive greater significance upon relating the distribution of lethals to the distribution of the break points involved in the interchanges and to the nature of the compound autosomes from which the detachment products were obtained.

**Detachment products of C(2L)SH3,+:C(2R)SH3,+:** Before examining the results of Experiment 1, let us consider the consequence of an interchange between a C(2L) and a C(2R) which carry neither duplications nor deficiencies for proximal genetic loci. Such a pair of compound autosomes is diagrammed in Figure 1. Consider that the markers a b on C(2L) and c d on C(2R) indicate the normal sequence corresponding to the standard second from which the compounds were generated. Consider, further, that deficiencies for these loci are recessive lethals (or possibly recessive visibles) but that these loci fall within a region for which partial trisomy has little or no apparent effect on viability.

If we view the recovery of reconstituted standard seconds as one half the products of a translocation (or interchange) between radiation induced chromatid breaks on C(2L) and C(2R) and, moreover, if we consider that within any series breaks are randomly distributed between or adjacent to the markers a and d on C(2L) and C(2R) respectively, a population of interchange products will be heterozygous for varying degrees of segmental aneuploidy (i.e. duplications and deficiencies) in the proximal left and right arms. In Figure 1 the left arm of chromosome-2 is
The frequency of homozygous lethality associated with chromosomes recovered as detachment products from three different strains of gamma-radiation treated compound-2 females.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Compound autosomes</th>
<th>Total detachments tested</th>
<th>Homozygous lethals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C(2L)SH3, +;</td>
<td>83</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>C(2R)SH3, +.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>C(2L)SH3, +;</td>
<td>110</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>C(2R)VKl, Dp(2L)lt^bw/bw.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C(2L)SHl, Dp(2R)rl^+/+;</td>
<td>60</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>C(2R)SH3, +.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FIGURE 1

Hypothetical model depicting representative genotypes of reciprocal detachment products expected from interchanges between $C(2L)$ and $C(2R)$ chromosomes that carry neither deficiencies nor duplications for proximal genetic loci.
Regions of Interchange

Interchange Products

Genotype

3 - 4

\[ a \quad b \quad K-2L \quad c \quad d \]

Normal

3 - 5

\[ a \quad b \quad K-2R \quad c \quad d \]

Dup(2R)c

\[ a \quad b \quad K-2L \quad d \]

Def(2R)c

2 - 4

\[ a \quad b \quad K-2R \quad b \quad c \quad d \]

Def(2L)b

1 - 5

\[ c \quad K-2R \quad c \quad d \]

Dup(2L)ab: Def(2R)c

Def(2L)ab: Dup(2R)c
divided into three regions as defined by the markers a and b, with region 3 designating that segment of the chromosome between the most proximal locus and the centromere (K-2L) of C(2L). The right arm is similarly divided into three regions with 4 designating the most proximal segment. The lower portion of Figure 1 depicts representative interchange products one might anticipate when the acentric fragment from one compound fused with the centric free arm of the complementary compound autosome. When the interchange occurs between breaks in regions 3 and 4, the reciprocal products will differ only in the source of the centromere (either K-2L or K-2R) and should be homozygous viable. If, however, the break is proximal in one compound autosome, for example region 3 in C(2L) and distal to a functional locus in the other (in this example compound-2R) the interchange product carrying the C(2L) centromere (K-2L) will be deficient for the proximal marker in 2R, but the reciprocal product, with K-2R, will carry the corresponding duplication. Similar duplication-deficiency interchange products arise from a distal break in C(2L) and a proximal break in C(2R) (e.g. regions of interchange 2-4 in Figure 1). Finally, if the interchange occurs between induced breaks distal to functional loci both in C(2L) and in C(2R) (for example, regions 1 and 5 in Figure 1) the K-2L product will be duplicated for the proximal left arm and deficient for the proximal right, while the K-2R product will carry the reciprocal combination of duplication-deficiency.

In accordance with the above model, which considers compound autosomes that are isochromosomes in so far as they are diploid for all functional genetic loci, the interchange products will fall into three separate groups: 1) homozygous viables, 2) homozygous lethals owing to a
deficiency in 2L proximal and 3) homozygous lethals owing to deficiencies in 2R proximal. Interchange products deficient both for 2L and 2R loci will not arise from compound autosome detachment where both compounds are fully diploid; they should only occur where at least one compound autosome is hemizygous for proximal loci. Furthermore, deficiencies are polar, that is they extend from the centromere distally and always include the most proximal locus. For example, in Figure 1, deficiencies in proximal 2L are either b or a b, but not a alone.

The distribution of lethals (and semi-lethals) obtained in the detachment products of the C(2L)SH3,+;C(2R)SH3,+ chromosomes (Figure 2) is consistent with the predictions of the above model. Of the 83 detachment products recovered as fertile individuals, 28 were homozygous complete lethals and 8 were classified as semi-lethals. On the basis of inter se complementation, allelism tests with known recessive lethals and lethal deficiencies within the proximal region, and pseudodominance tests against visible markers within this region (see Table I and Figure 2), 35 of the lethals, which includes all 8 semi-lethals, were localized to two main clusters, one on either side of the centromere. The remaining single lethal, which gave full complementation with all others, probably arose from a secondary hit as it falls well outside the proximal heterochromatic region. It is of interest to note that the yield of lethal detachments (less than 50 percent) and the absence of double lethals (i.e. lethals spanning the centromere) argues that neither C(2L)SH3,+ nor C(2R)SH3,+ is hemizygous for proximal loci. In this context it is interesting to note that both C(2L)SH3,+ and C(2R)SH3,+ appear as isochromosomes in both polytene and somatic chromosome preparations.
Genetic map of the centric region of chromosome-2 showing the relative positions and lengths of proximal deficiencies associated with reconstituted standard seconds generated as products of interchange between C(2L)SH3,+ and C(2R)SH3,+. The marker positions shown on the map are only relative and do not represent actual physical separation. Genetic markers contained in ] represent putative positions of recessive lethals defined by the newly generated deficiencies. Proximal heterochromatin is indicated by X\NNW. The position of Minute (M-S2) relative to the heterochromatic-euchromatic junction is not known. The dotted line under Group E indicates the position, relative to 1t, of the lethal associated with the 2;3 quasi-reciprocal translocation. See text for further details.
In addition to lethality tests, each detachment product was genetically tested for \( X;2, 2;3 \) and \( 2;4 \) translocations. Although rarely expected multiple-hit events, one such product was recovered and will be described below.

The nature of the proximally located lethals, depicted in Figure 2, is based on the concept that detachment products, as a function of compound interchange, will carry deficiencies (and duplications) of varying lengths. Focusing attention first upon the \( 2R \) associated lethals we find that all 18, which represents one half of all lethal detachments recovered, are lethal over the proximal deficiency, \( \text{Df}(2R)M-S2^{10} \).

Deficiency \( M(2)S10 \) is defined genetically as a recessive lethal with a dominant Minute phenotype (see Lindsley and Grell 1968) and which also uncovers the more proximal marker, rolled (\( rl \)); cytologically, \( M(2)S10 \) is apparently deficient for only the most proximal three polytene chromosome bands in \( 2R \) but it reduces the mitotic metaphase length of \( 2R \) to approximately \( \frac{3}{4} \) of its normal size (Morgan, Schultz and Curry 1940). It has also been reported to behave as a heterochromatic deficiency in that it enhances position-effect variegation (Morgan et al. 1940, Hannah 1951). While I have been unable to confirm the absence of the proximal bands (in 40A) of the polytene, mitotic prometaphase preparations of \( M(2)S10 \) obtained (see Figure 3) clearly show that the proximal \( 2R \) heterochromatin is missing.

The detachment lethals in \( 2R \) can be subdivided into two groups. Group A mutants, of which there are 17, in addition to being lethal over \( \text{Df}(2R)M-S2^{10} \), uncover the marker \( rl \). The distal boundaries of these deficiencies can be defined further as they complement the Minute
FIGURE 3

Photomicrograph of a stained preparation of mitotic prometaphase chromosomes in a ganglion cell from a $+/\text{Df}(2R)\text{M-S2}^{10}$ heterozygous, third-instar, male larva. The dotted arrow points to the $2R$ proximal heterochromatin on the $+$ chromosome; the solid arrow points to the $\text{M(2)S10}$ ($2R$ heterochromatic-deficient) chromosome.
deficiencies, M(2)S4 and M(2)S8. The latter two mutants are not only lethal over M(2)S10 but, genetically, extend distally to include the markers indicated on the map in Figure 2. The single Group B lethal, which is lethal over Group A and M(2)S10, does not uncover rl. Only if C(2L)SH3,+ carried a rl* duplication, which it does not, could the Group B lethal locus (or loci) fall between rl and the M(2)S2 site. Therefore, the conclusion is that Group B represents a deficiency that uncovers a genetic locus (or loci) proximal to rl.

Turning next to the 2L associated lethals, we can assign relative map positions to 17 lethals (and semi-lethals) on the basis of inclusion within the Group C deficiencies. The Group C deficiencies, of which there were two, uncover light (lt), the most proximal genetic marker heretofore localized on 2L. The six Group D lethals, which fail to complement with each other and with the Group C lethals, do not include lt. Therefore, consistent with the argument presented for the positioning of Group B on the right arm, we envisage Group D to represent deficiencies in 2L that include functional genetic loci (or a single locus) proximal to lt. The 8 semi-lethals, denoted by Group D' in Figure 2, lead us to suspect that Group D deficiencies do, in fact, uncover more than a single genetic site. The 8 semi-lethals have in common the following characteristics: 1) their viability, relative to heterozygotes, is between 5 and 15 percent; 2) those that eclose are greatly reduced in size (to approximately 1/2 the size of a normal adult); and 3) they eclose very late. Furthermore, all 8 Group D' semi-lethals manifest these same characteristics over any of the Group D or Group C (lethal) deficiencies. While one might be tempted to interpret Group D and
Group D as representing deficiencies of varying lengths extending into a tandemly replicated series of genes, somewhat analogous to the bobbed mutations of the X-chromosome. I view this as unlikely for two reasons. 1) The viability and phenotypic expression of all homozygous Group D deficiencies are manifestly the same as heterozygous Group D'/Group C deficiencies. 2) EMS (ethylmethane sulfonate) mutagenic studies (Chapter IV) reveal at least two loci within Group D, one of which expresses the D' mutant characteristics. I propose, therefore, that the present deficiency mapping has disclosed at least two genetic loci within the heterochromatic region between 1R and the centromere.

The single Group E recessive lethal, represented as a dotted line in Figure 2, though genetically behaving as a nonpolar deficiency, is in fact a C(2L)-3-C(2R) quasireciprocal translocation. This arose from a 3-hit event that translocated 2L, including a large block of heterochromatin proximal to the 2L secondary constriction, to the right arm of chromosome-3 at polytene band 92E-F of Bridges' map (see Lindsley and Grell 1968). The right arm of chromosome-3 distal to 92E-F is appended to the centromere-bearing detachment of C(2R). While this 2;3 translocation is lethal over Group C deficiencies, it is fully viable over all Group D deficiencies. Furthermore, flies heterozygous for Group E over In(2LR)bwV1, an inversion with a breakpoint in the left proximal heterochromatic region, are complete lethals and Group E over 1R shows the classical position-effect variegation for light eyes (Schultz 1936). The rare homozygous Group E translocation progeny that emerge as adults (approximately 1.0 percent of the heterozygotes) are phenotypically 1R variegated. It is interesting to note that since the
translocation is not lethal over Group D deficiencies, the break in 2L must lie proximal to the Group D lethals. Moreover, the position-effect variegation, owing to \( \text{lt}^+ \) being displaced from the centric heterochromatin to a euchromatic region, does not affect the more proximal loci sufficiently to cause lethality. (See Appendix I for further discussion of this unusual 2;3 translocation and its relevance to \( \text{lt} \) variegation.)

Turning now to the distribution of the break points involved in the compound-compound interchanges, let us examine the following arguments. Considering the proposed model for reconstituting standards from compound autosomes, approximately one half of the detachment products carried an acentric right arm and half carried an acentric left. From the 83 detachments recovered, 18, or approximately 1/4, are deficiencies of proximal 2R and 17, again approximately 1/4, are deficiencies of proximal 2L. Considering first the 2R deficiencies, we find that 17 of the 18 lethals uncover \( r_1 \) but none uncover the Minute lethal defined by the overlapping of deficiencies \( M(2)S10 \) with \( M(2)S4 \) and \( M(2)S8 \). Furthermore, as shown above, \( M(2)S10 \) is apparently deficient for all proximal 2R heterochromatin. If we can correctly assume that break-points occur at random within the heterochromatric region, we are led to the conclusion that \( r_1 \) is a gene intercalated approximately midway between the centromere and the heterochromatic-euchromatic junction. The Group B deficiency I view as revealing at least one additional locus proximal to but probably quite tightly linked to \( r_1 \).

For the left arm I can offer a similar argument. I have found that \( C(2R)VK1,Dp(2L)lt^+ ,bw \) (the chromosome in Experiment 2 described below) which carries a \( \text{lt}^+ \) duplication, does not possess the prominent
secondary constriction frequently observed at the euchromatic-heterochromatic junction of 2L (Kaufmann 1934). However, the prometaphase figures of the Group C (lt) deficiency-chromosomes do show this prominent secondary constriction. This places lt in the heterochromatic segment proximal to the constriction. The remaining Group D and Group D' deficiencies uncover genetic sites that are probably intercalated somewhere midway between lt and the centromere. Interestingly, the Group C deficiencies are not deficient for any proximal bands of the 2L polytene chromosome.

**Detachment products of C(2L)SH3, +; C(2R)VK1, Dp(2L)lt+, bw**: Let us first examine (in Figure 4) the consequence of interchanges between a C(2L) that is genetically an isochromosome and a C(2R) that carries a duplication for a proximal segment of 2L. Markers a b and c d represent the normal sequence in proximal 2L and 2R respectively. In addition, one arm of C(2R) carries a duplication for marker b intercalated between marker c and the centromere, K-2R. The designated regions 1, 2 and 3 of 2L in Figure 4 correspond to those described in Figure 1 above. However, on 2R, region 4 is homologous to region 3 on 2L; region 5 now designates the heterochromatic segment that normally separates the marker c and the centromere on the standard chromosome and region 6 corresponds to region 5 in Figure 1 above.

The lower portion of Figure 4 depicts some of the detachment products expected following interchanges between the designated regions on C(2L) and C(2R). The predictions for the recovery of deficiency products in the previous model (Figure 1) hold true for the products expected in Figure 4, with two major differences. If the points of induced breakage
FIGURE 4

Hypothetical model depicting representative genotypes of reciprocal products expected from interchanges between a $C(2L)$ that is genetically an isochromosome and a $C(2R)$ that is heterozygous for a duplication of the most proximal segment in 2L (indicated by the marker $b$).
Regions of Interchange

<table>
<thead>
<tr>
<th>Regions</th>
<th>Interchange Products</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 - 4</td>
<td>a b K-2L b c d</td>
<td>Dup(2L)b</td>
</tr>
<tr>
<td>3 - 5</td>
<td>a b K-2L c d</td>
<td>Normal</td>
</tr>
<tr>
<td>3 - 6</td>
<td>a b K-2R c d</td>
<td>Normal</td>
</tr>
<tr>
<td>3 - 6</td>
<td>a b K-2L c d</td>
<td>Dup(2L)b</td>
</tr>
<tr>
<td>2 - 5</td>
<td>a b K-2R c d</td>
<td>Dup(2L)b</td>
</tr>
<tr>
<td>1 - 5</td>
<td>b K-2R c d</td>
<td>Def(2L)c</td>
</tr>
</tbody>
</table>

Genotype

Dup(2L)b
Normal
Dup(2L)b
Dup(2L)b:(2R)c
Dup(2L)b
Dup(2L)ab
Def(2L)a
are randomly distributed, then 1) the proportion of \( b \) (or left arm) deficiencies will be markedly reduced and 2) nonpolar deficiencies of proximal 2L will be recovered (e.g. deficiency \( a \), but not \( b \), from interchanges at regions 1-5 in Figure 4).

From the analysis of detachment products in Experiment 1, the C(2L)SH3,+ chromosome was found to be an isochromosome for 2L free of a duplication for 2R genetic loci. The C(2R)VK1,Dp(2L)lt+,bw chromosome, in contrast, has been shown, in combination with a C(2L) homozygous for lt, to carry a 2L duplication for the lt+ gene. From this we may infer that the lt+ duplication on C(2R)VK1 includes the more proximal loci uncovered by the Group D deficiencies in Experiment 1.

In Experiment 2, of the 110 compound-detachment products 27 were homozygous lethals. As shown in Figure 5, 17 of these lethals were included within the M(2)S10 deficiency of proximal 2R, while only 5 detachment lethals were uncovered by the Group C deficiencies in proximal 2L. Of the remaining 5 homozygous lethals, one was lost prior to analysis and, through crossover studies, the remaining four mapped well outside the proximal region. These findings are consistent with the prediction of a relative reduction in generating deficiencies in 2L.

Directing attention first to the 2R associated lethals, we note that 16 of the 17 deficiencies are typically of the Group A class; that is they are lethal over M(2)S10 as well as Group A and Group B deficiencies and uncover the marker rl. The remaining 2R deficiency (designated as Group A' in Figure 5), in addition to revealing the Group A characteristics, is lethal both with M(2)S4 and M(2)S8. However, chemical mutagenesis studies (Chapter III) reveal a locus common to M(2)S10, M(2)S4 and M(2)S8 that is not included within the Group A' deficiency, demonstrating
Genetic map of the centric region of chromosome-2 showing the relative positions and lengths of proximal deficiencies associated with reconstituted standard seconds generated as products of interchange between C(2L)SH3,+ and C(2R)VK1,Dp(2L)1tbw/bw. 1(2)A' may be an allele of M-S2. Their separation on this map is to indicate that Df(2R)M-S210 uncovers at least one (recessive lethal) site that is not included within the Group A' deficiency. A more detailed description is given in the legend of Figure 2.
l(2) crc
tri
Secondary Constriction
\[ l(2L) F \]
l1
D'(3)
\[ l(2L) D' \]
Centromere
A(16)
A'(17)
rl
MS2-10
\[ l(2R) A \]
MS2
MS2-8
MS2-4
stw
ap
that the Group A' deficiency does not extend to the distal boundary of M(2)S10. The absence of Group B deficiencies in this experiment lends support to our previous assumption of tight linkage between the Group B lethal and the rl locus.

The distribution of (deficiency) lethals localized proximally in 2L clearly supports the prediction of recovering nonpolar deficiencies when C(2R) is heterozygous for a duplication of proximal 2L loci. The three lethals designated Group D' in this experiment were homozygous complete lethals but heterozygous combinations of these Group D' lethals as well as any D' in combination with Group C or Group D lethals (from Experiment 1) express similar properties to the Group D' lethals in Experiment 1. There were no Group C lethals per se but the two Group F lethals are apparently representative of the Group C class in arising from break points distal to the lt locus. The Group F deficiencies provide a further subdivision of the Group C deficiencies, obtained in Experiment 1, into a Group C' that is lethal over both Group F deficiencies and a Group C', whose distal break point must lie between lt and the Group F lethals.

Since the Group C' deficiency chromosome retained the prominent secondary constriction (loc. cit.), it would appear that the Group F deficiencies uncovered an additional locus (or loci) within the heterochromatic segment distal to the lt gene. Furthermore, the Group F deficiencies weakly complement the quasi-reciprocal 2;3 translocation (Group E lethal in Figure 2) and In(2LR)bw1, both of which show position effect variegation for light and are lethal over Group C and C' deficiencies. This points to the possibility of an additional genetic locus (or loci) between lt and the Group D deficiencies that is uncovered by Group C.
and C' and is primarily responsible for the position-effect lethality. Further, the Group F deficiencies are not deficient for any proximal bands in the 2L polytene chromosome.

**Detachment products of C(2L)SH1,Dp(2R)rl1+/+;C(2R)SH3,+**: As a parallel to Experiment 2 (above), I examined detachment products from a C(2L) that carried a duplication for proximal 2R, including the rl1+ gene. Regrettably, 59 of the 60 reconstituted standard seconds recovered were homozygous lethal. With the prior knowledge (from Experiment 1) on the genetic properties of the C(2R)SH3,+ chromosome, I viewed the high lethality rate as evidence for heterozygosity of preexisting lethals on C(2L)SH1. It is conceivable that, as a function of formation, C(2L)SH1 was heterozygous for a proximal deficiency on the same arm as the 2R(rl1+) duplication and at a more distal site, but close to the centromere, a recessive lethal on the homologous arm. Since the frequency of homozygosis near the centromere is low, this heterozygous combination of recessive lethals would remain relatively stable. Detachment products from the present experiment were not analyzed further. Nonetheless, this experiment served to emphasize the importance to this type of study for independent examination of homogeneous populations of compound autosomes.

The results from both Experiments 1 and 2 are summarized in Figure 6. Cytological observations place all of these deficiencies within the proximal heterochromatic regions of the left and right arms of chromosome-2. These regions represent approximately 25 percent of the physical length but only 0.1 percent of the genetic length of the entire chromosome (see Lindsley and Grell 1968). The present method of dissecting the proximal
FIGURE 6

Compilation of the genetic maps given in Figures 2 and 5.
heterochromatin does not resolve completely the debate concerning the nature of the genes intercalated into this region. In particular, deletion mapping cannot provide a clear distinction between possible tandemly repeated genes and single gene loci. However, it does demonstrate that within this segment of the chromosome, where DNA sequences (Botcham et al. 1971, Gall et al. 1971, Peacock et al. 1973) and cytological properties imply primarily genetic inactivity, active genetic sites do reside, albeit at low density. Moreover, the present results provide an estimate of the number of functional loci and their relative distribution in the heterochromatic region of chromosome-2. According to chemical mutagenesis studies each site inferred from the present deficiency analysis represents a nonrepetitive cistron. Furthermore, within the M(2)S10 deficiency, saturation mutagenesis studies reveal only three loci in addition to the three shown by the present study, thus demonstrating a low gene density in the 2R proximal heterochromatin.

Prior to this study, the markers 1t and r1 were defined as the most proximal genetic loci on 2L and 2R respectively. The proximal deficiencies generated through the detachment of compound autosomes reveal these markers as functional loci residing within cytologically defined heterochromatic segments. The frequency with which r1 and 1t are uncovered by deficiencies would suggest that r1 is more proximal in the 2R than is 1t in the 2L heterochromatic block.

Proximal to r1, on 2R, there is at least one additional vital locus defined by the Group B deficiency; and distal to r1, the lethal uncovered by the overlapping of Group A' with deficiencies M(2)S4 and M(2)S8 indicates a third heterochromatic locus. However, since M(2)S2 is located near the
heterochromatic-euchromatic junction, a region that cytologically is extremely difficult to resolve, I cannot discount the possibility that the distal lethal locus (uncovered by Group A') falls just outside the proximal heterochromatic segment.

Between the centromere and \( \mathbf{l} \mathbf{t} \) on \( 2L \), two functional loci are indicated by the differing phenotypic expressions of the Group D and Group D' deficiencies. Position-effect lethality suggests a possible third locus between Group D and \( \mathbf{l} \mathbf{t} \) (not indicated in Figure 6). Distal to \( \mathbf{l} \mathbf{t} \), as defined by the Group F deficiencies, at least one additional locus is recognized as residing within the heterochromatic segment of the chromosome. Hence, on the basis of deficiency mapping I propose, as a conservative estimate, that within the proximal heterochromatin of \( 2R \) there are at least two, and possibly three, functional genetic loci, including \( \mathbf{r} \mathbf{l} \), and within \( 2L \) at least four, including \( \mathbf{l} \mathbf{t} \). At the same time, I realize that less than half of the interchanges generated lethal deficiencies. Accordingly, if I am correct in assuming a random distribution of induced breaks throughout the heterochromatic regions and an equal probability of recovering acentric left and right arm detachments, I am led to infer genetic quiescence in the most proximal half of the centric heterochromatin of chromosome two.
CHAPTER III

ANALYSIS OF EMS INDUCED LETHAL ALLELES OF SECOND CHROMOSOME PROXIMAL DEFICIENCIES
INTRODUCTION

The previous chapter described a study in which the proximal region of chromosome-2 was genetically dissected by employing compound second autosome-detachments. In order to supplement and further this analysis I turned next to the induction of lethal alleles of chromosome-2 proximal deficiencies through the use of the chemical mutagen, EMS (ethyl methane sulphonate). In this fashion I could determine if loci, in addition to those identified by the detachment analysis, existed in the second chromosome heterochromatin. Further, by complementing the EMS induced lethals to the deficiencies described in the previous chapter, it was possible to demonstrate the relative map positions of the new loci uncovered.

This chapter describes the results of the analysis of EMS induced lethal alleles of the deficiencies, Df(2L)Group C' and Df(2R)M-S2\textsuperscript{10}, described in Chapter II. Df(2L)Group C' is the largest 2L proximal deficiency recovered by compound second autosome detachment. Df(2R)M-S2\textsuperscript{10} is deficient for the 2R heterochromatin.
MATERIALS AND METHODS

Generation of lethal alleles of Df(2R)M-S2\textsuperscript{10}:

A stock isogenic for chromosome-2 and homozygous for the second chromosome dominant mutation Pin was derived. Pin/Pin (iso-2) virgin males aged 2 to 3 days (posteclosion) were fed for 24 hours on a solution of 0.025 M EMS in 1% sucrose (Lewis and Bacher 1968). The males were then removed from the treatment vessels, placed in bottles containing a standard Drosophila medium and allowed to recover for 24 hours. The treated males were then mated to In(2LR)bw\textsuperscript{VI}/In(2LR)SM1 virgin females, with 1 treated male and 10 virgin females per culture. F1 male progeny heterozygous for the treated paternal chromosome (Pin) and In(2LR)SM1,Cy were single pair mated in shell vials to Df(2R)M-S2\textsuperscript{10}/In(2LR)SM1 virgin females. F2 cultures in which all progeny were of the Cy phenotype were scored as putative lethal alleles of Df(2R)M-S2\textsuperscript{10}. The presence of Pin on the treated chromosome allowed one to derive from the lethal cultures a balanced stock heterozygous for the putative lethal allele of Df(2R)M-S2\textsuperscript{10} and In(2LR)SM1,Cy.

Each putative lethal allele of Df(2R)M-S2\textsuperscript{10} was subsequently tested for complementation with the 2R proximal deficiencies described in Chapter II. Those putative lethal alleles of Df(2R)M-S2\textsuperscript{10} that proved to be lethal alleles of the M(2)S10 deficiency, rather than lethal alleles of secondary lethals accumulated by and distributed on the M(2)S10 chromosome, were complemented in all inter se combinations.
As all of the proximal lethal and deficiency stocks were heterozygous for \textit{In(2LR)SM1,Cy}, complementation was indicated by the presence of transheterozygote, non-\textit{Cy} progeny, whose phenotype was carefully examined. Complementation results of key importance in determining the nature of the often complex allele complementation maps were reconfirmed.

A control experiment was done for the \textit{Df(2R)M-S2\textsuperscript{10}} lethal allele screen; and the \textit{X}-chromosome spontaneous lethal rate was assayed in the \textit{iso-2 Pin} strain.

All experiments were performed at 25°C and employed a standard, cornmeal-agar-yeast-sucrose-dextrose Drosophila medium.

\textbf{Generation of lethal alleles of Df(2L)Group C':}

EMS induced lethal alleles of \textit{Df(2L)Group C'} were isolated and analysed by following the same procedures employed for the recovery of EMS induced lethal alleles of \textit{Df(2R)M-S2\textsuperscript{10}}. \textit{2L} rather than \textit{2R} proximal deficiencies were utilized in subsequent analysis to further characterize the newly induced recessive lethal (and visible) mutations.
RESULTS AND DISCUSSION

The spontaneous X-chromosome recessive lethal rate was determined in iso-2 Pin males. From 10 parental males, 1618 X-chromosomes were tested of which 4 were found to bear recessive lethals. (Of the 4 lethals, 2 were derived from a single male.) This spontaneous mutation rate, 0.25%, is well within the range observed for most Drosophila strains (Plough 1941). Thus the iso-2 Pin stock does not appear to be a highly spontaneously mutable strain.

Lethal alleles of Df(2R)M-S210:

In the control experiment, no lethal alleles of Df(2R)M-S210 were recovered in 1925 tested chromosomes. In the EMS treated series, 85 lethal alleles of Df(2R)M-S210 were recovered in 5000 fertile cultures, each fertile culture representing an individual tested chromosome. Unlike the control, appreciable sterility (approximately 16%) was observed in the F1 male progeny of the EMS treated iso-2 Pin males. In addition to the 85 lethal alleles of Df(2R)M-S210, 41 chromosomes were recovered which, although lethal when heterozygous with the M(2)S210 chromosome, were not lethal alleles of the M(2)S210 deficiency but, rather, were alleles of secondary lethals that the M(2)S210 chromosome had accumulated. The accumulation of lethals on permanently heterozygous second chromosomes has been well documented (Mukai 1964) and the M(2)S210 chromosome was constructed over 35 years ago (Morgan, Schultz and Curry 1940).

On the basis of complementation with the 35 available 2R proximal
deficiencies and the recessive mutation rl, the 85 lethal alleles of Df(2R)M-S2\textsuperscript{10} fell into five groups (Figure 1).

Let us now examine the complementation maps of each of the five sites, beginning with Group I. Group I lethals were lethal in combination with the Groups B, A, A' and A'' deficiencies, but they were rl\textsuperscript{+} and survived when heterozygous with Df(2R)M-S2\textsuperscript{4}. The complementation map of the Group I lethals is presented in Figure 2.

The Group I lethals can be divided further into two subgroups. One lethal, EMS 31, complements with all 4 remaining lethals. The other lethals, EMS 45-10, EMS 45-84, EMS 45-87 and EMS 45-91, provide a circular complementation map. This circular complementation map is clearly an example of interallelic complementation (reviewed in Fincham 1966). Obviously with such complex allele complementation occurring at the EMS 45-10 locus it is not possible on the basis of the complementing of EMS 31 with all 4 other Group I lethals to assign it to a different locus. However, observations on the phenotype of Group B deficiency homozygotes and heterozygotes with the Group I and the Groups A, A', and A'', and M(2)S2\textsuperscript{10} deficiencies strongly imply that EMS 31 represents a locus separate from the one associated with the EMS 45-10 complex.

Zygotes homozygous for the Group B deficiency or heterozygous for the Group B deficiency and a larger 2R deficiency, which encompasses the Group B deficiency, survive to the third larval instar but do not pupate. These nonpupating larvae develop extremely large melanotic masses in their haemocoel. Virtually all of the deficiency homozygote larvae develop these melanomas and, usually, when they die, they contain one or more very large, and numerous small, melanomas (Figure 3). Heterozygotes
FIGURE 1

Distribution by complementation with 2R proximal deficiencies of EMS-induced lethal alleles of Df(2R)M-S210. Proximal heterochromatin is indicated by \_\_\_\_\_. See text, and Figures 2, 5, and 6 of Chapter II, for further details.
FIGURE 2

Complementation map of the Group I lethal alleles of $Df(2R)M-S2^{10}$. 
FIGURE 3

Photograph of a third-instar larvae homozygous for the Group B 2R proximal deficiency.
for EMS-31 and any of the Groups A, A', A", and B or M(2)S10 deficiencies have the Group B syndrome, being late larval lethals with the lethal larvae developing huge melanotic masses. None of the 4 lethals belonging to the EMS 45-10 complex exhibit the Group B (melanotic) syndrome. However, all are late larval and pupal lethals. Thus, it would appear that the Group I lethals represent two loci, one represented by one lethal, EMS 31, and the other represented by four, EMS 45-10, EMS 45-84, EMS 45-87, and EMS 45-91. As both sets of Group I lethals are lethal in combination with the Group B deficiency and are rl+, it is clear that both loci are proximal to the rl locus. Their relative order is unknown and failing the recovery of a proximal deficiency whose distal or proximal boundary falls between the two loci, this will be resolved only by conventional genetic mapping. However, it will probably be difficult, if not impossible, to separate these two sites through recombination, as indicated by the following observation.

A recombination experiment between EMS 31 and rl was attempted. Virgin females heterozygous for EMS 31 and b pr rl cn (see Table I of Chapter II for a description of these mutations) were crossed to Df(2R)M-S210/In(2LR)SM1 males. As rl is virtually lethal when hemizygous at 25°C (the rl hemizygotes have less than 1% viability and the rare surviving rl hemizygote has a very severe rl phenotype) the only regular surviving progeny are In(2LR)SM1 heterozygotes which have the Cy wing phenotype. A Cy+ exception could arise as a result of recombination between rl and EMS 31. However, such a recombinational event has only a 25% chance of being recovered as a Cy+ exception for it requires that the EMS 31+ rl+ recombinant chromatid be selected for in a
female pronucleus that is fertilized by a Df(2R)M-S2\(^{10}\) bearing sperm. As the total number of zygotes is equal to approximately twice the number of Cy progeny, one may estimate the recombination frequency as two times the number of recombinants divided by the number of Cy progeny. No recombinants between EMS \(_{31}\) and \(_{rl}\) were observed in the 13,268 progeny recovered. Clearly, as the 95% confidence limits (Stevens 1942) for the recombinational distance between EMS \(_{31}\) and \(_{rl}\) are 0 and 0.045%, it may well be very difficult to order EMS \(_{31}\) and the EMS \(_{45-10}\) complex by recombination.

The Group II lethals are all alleles of the \(_{rl}\) locus (see Figure 4 for the complementation map). All 9 Group II lethals are lethal when heterozygous with the Groups A, A', and A'' deficiencies but survive in combination with the Group B deficiencies as well as with the M(2)S2\(^{4}\) deficiency and the Groups I, III, IV, and V EMS lethals. Eight of the Group II lethals, EMS \(_{43}\), EMS \(_{64}\), EMS \(_{34-29}\), EMS \(_{45-32}\), EMS \(_{45-39}\), EMS \(_{45-54}\), EMS \(_{45-95}\) and EMS \(_{698}\) are lethal when heterozygous with the original \(_{rl}\) mutation; one Group II lethal, however, EMS \(_{45-52}\), while extremely inviable when homozygous, when heterozygous with \(_{rl}\) is associated with a fully viable fly expressing a phenotype indistinguishable from that of the original \(_{rl}/_{rl}\) homozygotes. Additionally, all 9 Group II lethals are lethal in all inter se combinations. Interestingly, the Group II lethals, save for EMS \(_{45-52}\), when hemizygous (i.e. heterozygous with a \(_{rl}\) deficiency), or in any inter se combination, are associated with a lethal phenotype wherein the organisms develop into third instar larvae that completely lack imaginal discs.
FIGURE 4

Complementation map of the Group II lethal alleles of

\( \text{Df(2R)M-S2}^{10} \).
EMS 43
EMS 64
EMS 698
EMS 34-29
EMS 45-32
EMS 45-39
EMS 45-52
EMS 45-54
EMS 45-55
The complementation map of the Group III lethals is presented in Figure 5. The Group III lethals divide the previously described Group A deficiencies (Chapter II) into two classes, Group A deficiencies that do not include the Group III lethals, and Group A" deficiencies that do. The 6 Group III lethals, EMS 34-7, EMS 45-1, EMS 45-17, EMS 45-37, EMS 45-40, and EMS 45-73 do not complement in any intersect combination and thus would definitely appear to be associated with a single locus. Of the 33 compound-second autosome detachment-associated deficiencies that uncovered r1, 21 include the EMS 34-7 locus.

Results from compound-autosome detachment studies indicate that all breaks in C(2R) occurred proximal the heterochromatic-euchromatic junction. Since approximately one-half of the 2R heterochromatic deficiencies included the marker r1, the physical location of this locus was estimated to be near the middle of the heterochromatic segment. Following this same line of argument, the inclusion of the EMS 34-7 locus in slightly more than one half the r1 deficiencies would place EMS 34-7 just proximal to the distal quarter of the heterochromatic region.

However, despite the appreciable physical distance that separates the two loci, roughly 1/6 to 1/4 of the 2R heterochromatic block, there is virtually no recombination between r1 and EMS 34-7. Virgin females heterozygous for EMS 34-7 and b pr rl cn were mated to Df(2R)M-S210/In(2LR)SM1 males. From this cross, no recombinant Cy+ progeny were recovered in 20,750 Cy progeny examined. This gives an upper 95% confidence limit of 0.029% crossing over between the two heterochromatic loci.
FIGURE 5

Complementation map of the Group III lethal alleles of $\text{Df(2R)M-S}^2_{10}$. 
EMS 34-7
EMS 45-1
EMS 45-17
EMS 45-37
EMS 45-40
EMS 45-73
The Group III lethals, when hemizygous, usually die in the late pupal stage with many dying while eclosing. The rare hemizygous survivors have unexpanded wings and, often, misshapen rear legs, etched tergites, and smaller body size.

The next group, the Group IV lethals, present a rather complex complementation map (Figure 6). In addition to \( \text{Df(2R)M-S2}^{10} \), the Group IV lethals are lethal in combination with \( \text{Df(2R)M-S2}^{4} \) and the single Group A' lethal. As the Group IV lethals fall within the \( \text{M(2)S2}^{4} \) deficiency, a deficiency for which I can detect neither loss of 2R proximal heterochromatin in somatic chromosome preparations nor deficiency for any proximal bands of the 2R polytene chromosome, the locus associated with the Group IV lethals is probably in the vicinity of the heterochromatic-euchromatic junction. Thus it is unclear whether the Group IV locus is within or immediately adjacent to the 2R proximal heterochromatin. The recovery of only one compound-second autosome detachment deficient for the Group IV site is further evidence for the location of the Group IV locus near the heterochromatic-euchromatic junction.

A total of 35 recessive lethals were recovered for the Group IV site. Of these lethals, 34 fell into an extensive and complex interallelic complementation map (Figure 6), while the remaining lethal, EMS 45-72, complemented with all others in this group. The complementation between alleles was surprisingly unambiguous, that is, heterozygous combinations of different alleles were either fully viable or completely inviable. Although not thoroughly studied, the lethal phases for the majority of homozygous and heterozygous combinations appeared to fall
FIGURE 6

Complementation map of the Group IV lethal alleles of Df(2R)M-S2^{10}. (EMS 45-72, not shown in the complementation map, complements fully with all other Group IV lethals).
into the late larval and early pupal stages of development.

The exceptions to complete lethality within the Group IV lethals involve 4 combinations which were associated with partial complementation, two combinations involving EMS 34-28 and two involving EMS 45-71. In Figure 6, EMS 34-28 is shown as noncomplementary to EMS 45-16 and EMS 45-28 while, in fact, it does complement weakly with both lethals. EMS 34-28/EMS 45-16 heterozygotes have 17% viability and EMS 34-28/EMS 45-28 heterozygotes have 8% viability relative to the In(2LR)SM1, Cy heterozygotes. EMS 45-71, on the other hand, is shown in Figure 6 as complementing with EMS 34-26 and EMS 45-34, even though it does so only weakly. EMS 45-71/EMS 34-26 heterozygotes have 10% viability and EMS 45-71/EMS 45-34 heterozygotes have 21% viability. These combinations are, however, the only ones which show ambiguity with respect to complementation.

Thus the Group IV lethals are associated with a locus that exhibits a relatively high rate of EMS mutability and whose lethal alleles exhibit complex interallelic complementation. The exceptional lethal in this group, EMS 45-72, may, in fact, represent an additional locus, but this is unclear.

The Group V lethals are also associated with a circular complementation map (Figure 7). The Group V lethals are lethal with none of the compound-second autosome detachment 2R proximal deficiencies, but are lethal in combination with Df(2R)M-S2^4 and Df(2R)M-S2^8. Moreover, they are viable in all combinations with the Group IV (and Groups I, II, and III) lethals. This places the locus associated with the Group V lethals distal to that associated with the Group IV. The Group V locus, therefore, falls near the 2R heterochromatic-euchromatic junction, but it is not clear whether
FIGURE 7

Complementation map of the Group V lethal alleles of $\text{Df(2R)M-S2}^{10}$. 
it lies just within or just outside the 2R heterochromatic block.

Complementation among the Group V lethals is relatively straightforward, 27 of the 30 lethals forming a uniformly noncomplementing complex. These 27 lethals are subdivided by their complementation with 3 exceptional alleles EMS 45-8, EMS 34-20, and EMS 45-89 which complement with most of the other alleles. Complementation is either complete or completely negative in all combinations of alleles save 4, 3 involving EMS 45-8 and 2 involving EMS 45-26. Although EMS 45-8 is shown in Figure 7 as noncomplementing with EMS 34-4, EMS 45-8/EMS 34-4 heterozygotes do have 8% viability. Further, although EMS 45-8/EMS 45-67 heterozygotes are indicated as complementing in Figure 7, these heterozygotes have 48% viability; which is considered to be essentially full complementation. Although EMS 45-26 complements fully with EMS 45-8 and EMS 34-20, the transheterozygotes have a peculiar imaginal external phenotype. EMS 45-26/EMS 45-8 heterozygotes have their wings uniformly spread out from the body at a 45° angle. EMS 45-26/EMS 34-20 heterozygotes have, in addition to the spread wing phenotype of EMS 45-26/EMS 45-8 heterozygotes, ocelli that are often misshapen, unpigmented, or absent.

The large number of Group V lethals recovered (a total of 30) points to a relatively high EMS mutability of the Group V locus. This is a characteristic it shares with the immediately adjacent Group IV locus in addition to interallelic complementation.

Recombinants were recovered between a Group V lethal and rl. Virgin females heterozygous for EMS 34-21 and b pr rl cn were crossed to Df(2R)M-S210/In(2LR)SMI males. 2 recombinant Cy+ progeny were recovered along with 5996 Cy progeny. That the putative recombinant
progeny were truly recombinant was verified by genetic testing. The 95% confidence limits (Stevens 1942) on the recombinational distance between rl and EMS 34-21 are 0.01 and 0.20 map units. Thus although the rl and Group V loci are separated by the distal half of the 2R heterochromatic block, crossing over between the two sites is less than 1%. The crossover distance between two markers flanking an equal extent of 2R euchromatin would be of the order of magnitude of 10 map units (Lindsley and Grell 1968).

The analysis of the EMS induced lethal alleles of Df(2R)M-S2\textsuperscript{10} has revealed the existence of 3 loci within the M(2)S10 deficiency in addition to the three demonstrated by the analysis of compound-second autosome detachments (Chapter II). Thus there are at least 6 loci within the M(2)S10 deficiency with at least 4 of these loci the EMS 31 locus, the EMS 45-10 locus, the rl locus and the EMS 34-7 locus, within the 2R proximal heterochromatin. The other two loci, the Groups IV and V loci, are near the 2R heterochromatic-euchromatic junction, but only one, Group V, falls outside the deficiencies generated through detachment of compound-2 autosomes.

Of the 85 EMS induced lethal alleles of Df(2R)M-S2\textsuperscript{10}, none are deficient for 2 or more loci; that is, all appear to be point mutations. This is consistent with the literature on EMS mutagenesis in Drosophila. Lim and Snyder (1974), in their analysis of 85 EMS induced lethals falling within the zeste-white region of the X-chromosome using the mutagenesis procedure of Lewis and Bacher (1968) that I have employed in my analysis of the proximal region of chromosome-2, found none that affected more than one cistron, even though this region is very well defined
genetically (Judd, Shen and Kaufman 1972). Earlier, Lim and Snyder (1968), in a cytological examination of EMS-induced X-chromosome recessive lethals, found that none of the lethals involved deletions, inversions or other visible rearrangements. Further, EMS is unable to induce, in Drosophila females, compound autosome formation (which, like the generation of deficiencies, is an interchange event) at doses capable of inducing a rate of X-chromosome recessive lethality of approximately 2%. (W. Gibson, personal communication).

It is unlikely that any of the Groups I and II lethals are deficiencies for it is clear from the detachment analysis that breaks are far more likely to occur distal to \( r_l \) and proximal to Group I than to occur between \( r_l \) and the Group I loci. Thus a deficiency for \( r_l \) is far more likely to be deficient for both \( r_l \) and the Group I loci than deficient for \( r_l \) only.

It is particularly unlikely that any of the Group IV or Group V lethals are deficiencies. The Group IV lethals are associated with a complementation map (Figure 6) in which no single lethal allele fails to complement all other lethal alleles in the group, as would be expected for a deficiency. As for the Group V lethals, a deficiency in this region would be far more likely to have its proximal break proximal to the Group IV locus than between the Group IV and V sites; and none of the Group V lethals are lethal in combination with any of the Group IV lethals.

Thus, the 85 lethals recovered that fall within the \( M(2)S2_{10} \) deficiency would appear to be point mutations implying that the loci they uncover are nonrepetitive. Moreover, the complex allele complementation
maps of the EMS 45-10, Group IV and Group V loci provide further evidence that the genetic loci uncovered in 2R heterochromatin represent single, nonrepetitive genes.

From the preceding analysis of compound second autosome detachments (Chapter II) and the recovery of EMS induced lethal alleles of Df(2R)M-10, it is clear that typical (i.e. nonrepetitive and viability-essential) loci exist within constitutive heterochromatin, although at very low density relative to euchromatin. The estimated relative gene density of the 2R heterochromatic block to an equal amount of 2R euchromatin is 1%, that is there is approximately one hundred times more genetic loci in a block of 2R euchromatin than there is in a block of 2R proximal heterochromatin of similar length. This estimate is based on the observation that the 2R polytene chromosome contains 1136 bands (Bridges and Bridges 1939), which would imply that a portion of 2R euchromatin equal in somatic chromosome length to the 2R heterochromatic block would be represented in the polytene chromosome by a 379 band segment. As there is substantial evidence (reviewed in Lefevre 1974) for the proposition that each polytene chromosome band is associated with a single genetic locus (Bridges 1935), this leads one to an estimate of approximately 380 genes in the 2R heterochromatic block. Within the 2R heterochromatin I have been able to identify 4 (and possibly 5, if Group IV is included) gene loci, thus the gene density of the 2R heterochromatin would appear to be only in the order of magnitude of 1% that of the estimated gene density of an equal extent of 2R euchromatin.
Lethal alleles of Df(2L)C':

EMS mutagenesis yielded 28 lethal alleles of Df(2L)C' in 6467 fertile cultures, each culture representing a single tested chromosome. In addition there was approximately 20 percent sterility (1591 of a total of 8058 cultures) in the F1 male progeny of the EMS treated iso-2 Pin males. In addition to the 28 lethal alleles of Df(2L)C', 5 alleles of It were recovered and were found to be viable over the C' deficiency, which, as shown in the previous chapter, is deficient for the It locus.

A phenomenon noted in the Df(2R)M-S210 lethal allele screen was also witnessed in this series of tests. Of a total of 6490 fertile cultures in the Df(2L)C' lethal allele screen, 23 had all Cy Pin+ progeny despite the fact that the paternal genotype was Pin/In(2LR)SM1. A possible explanation for these 23 exceptional cultures is that the male bearing the EMS treated iso-2 Pin chromosome was mosaic for a dominant lethal mutation on the mutagenized chromosome-2, the germ line bearing the dominant lethal mutation; the soma not bearing it. All of the F2 zygotes inheriting the paternal Pin chromosome (derived of course from the paternal germ line) would be heterozygous for the dominant lethal in all cells and, therefore, would die. These cultures were not numbered among the tested chromosomes, as I was unable to determine whether or not the paternal mutagenized Pin chromosome bore a lethal allele of Df(2L)C'.

On the basis of complementation with the 2L proximal deficiencies described in Chapter II, the 28 lethal alleles of Df(2L)C' fall into 4 groups (Figure 8), numbered VI, VII, VIII and IX to avoid confusion with the EMS lethal alleles of Df(2R)M-S210.
FIGURE 8

Distribution by complementation with 2L proximal deficiencies of EMS induced lethal alleles of Df(2L)C'. Proximal heterochromatin is indicated by \ldots, and the secondary constriction at the 2L heterochromatic-euchromatic junction by ( ). For further details see text and Figures 2 and 5 of Chapter II.
Let us now examine the complementation maps of each of the four groups of lethals beginning with the most proximal, Group VI (Figure 9). The Group VI lethals are lethal when heterozygous for the Groups C, C', D, and D' 2L proximal deficiencies but they are $lt^+$. The Group VI lethals, EMS 40-5 and EMS 56-19, were noncomplementing in combination but complemented fully when heterozygous for all of the Groups VII, VIII and IX lethals. Group VI lethal hemizygotes were like Group D' deficiency homozygotes distinguished by infrequent adult survivors who, although of a normal external phenotype, were late eclosing and greatly reduced in size (to approximately 1/2 the normal adult size).

The existence of the Group VII lethals confirms the previous inference that the Groups D and D' deficiencies were genetically distinct (Chapter II) as the Group VII lethals were lethal in combination with the Groups C, C' and D deficiencies but complemented fully with the Group D' deficiencies. Clearly, the Group D deficiencies have a greater distal extent than the Group D' deficiencies as was previously inferred (Chapter II). Inspection of the Group VII lethal complementation map (Figure 10) leads one to conclude that possibly two loci are associated with the Group VII lethals. EMS 56-24 complemented fully with the other 3 Group VII lethals. (EMS 56-24 hemizygotes were of approximately 2% viability.) The other subgroup consists of the EMS 56-4, EMS 56-14, and EMS 56-15 lethals. The lethals of the EMS 56-4 complex were completely inviable when hemizygous and in all inter se combinations of the three lethals. A semi-lethal allele of the EMS 56-4 locus was recovered, EMS 40-22, although this semi-lethal is not included in the Group VII complementation map illustrated in Figure 10. EMS 40-22 was of approximately 20% hemi-
FIGURE 9

Complementation map of the Group VI lethal alleles of \( \text{Df}(2L)C' \).
FIGURE 10

Complementation map of the Group VII lethal alleles of

Df(2L)C'.

zygous viability with the hemizygote progeny being late eclosing. Heterozygotes for EMS 40-22 and EMS 56-4, EMS 40-22 and EMS 56-14, and EMS 40-22 and EMS 56-15 were of 33, 26, and 35 percent viability respectively, with these heterozygous progeny being of later eclosion than their sibs. EMS 40-22 complemented fully with all other 2L lethals including EMS 56-24, the remaining Group VII locus. Thus it would appear that two loci are associated with the Group VII lethals, one locus with EMS 56-24 and the other with the EMS 56-4 complex, but their relative order is unknown.

The Group VIII lethals are uncovered only by the Group C and C' deficiencies and therefore define a region distal to the Groups D and D' deficiencies and proximal to the Group F deficiencies. Group VIII is divided into three distinct, nonoverlapping complementation subgroups (Figure 11), one of which corresponds to the 1t locus. In addition to 5 visible alleles of 1t, which were hemizygous viable and of 1t phenotype, 3 lethal alleles of the 1t locus were recovered, EMS 40-12, EMS 40-17 and EMS 56-3. These lethal alleles of 1t were of light phenotype when heterozygous for a nonlethal 1t allele but were lethal in combination with one another. Hemizygotes for the 1t lethal alleles appeared to die in the late pupal stage of development. Thus, it would appear that 1t is an essential locus. The two other complementation groups within the Group VIII lethals are the EMS 40-2 complex of 3 lethals and the EMS 40-6 complex of 14 lethals. The EMS 40-2 complex consists of EMS 40-2, EMS 56-6 and EMS 56-32. All 3 lethals of the EMS 40-2 complex are 1t+ and viable in combination with the 25 other Df(2L)C'
FIGURE 11

Complementation map of the Group VIII lethal alleles of Df(2L)C'.
<table>
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<th>EMS 40-2</th>
<th>EMS 40-6</th>
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<td>EMS 40-7</td>
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<td>EMS 56-27</td>
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</table>
lethal alleles. The significance of the fact that 14 of the 28 EMS induced lethal alleles of Df(2L)C' fall at this site is unclear, although it is interesting to note that, unlike the two loci in the proximal region of 2R associated with high EMS mutability, the EMS 40-6 complex is not associated with interallelic complementation. In summation, it would appear that the Group VIII lethals are associated with three loci, one corresponding to the It locus, for which in addition to 3 lethal alleles 5 hemizygous viable visible alleles were recovered; another, to the EMS 40-2 complex for which 3 lethal alleles were obtained; and the third locus, to the EMS 40-6 complex which represented 14 lethal alleles. The relative order of these three loci remains unknown.

The most distal 2L lethals are those associated with Group IX. The 2 Group IX lethals, EMS 40-18 and EMS 56-8, although lethal when heterozygous for the Group C' deficiency, are fully viable in combination with the Group C deficiency and are 1t+ . Both lethals are, however, lethal in combination with the Group F deficiencies; and EMS 40-18/EMS 56-8 heterozygotes are inviable, thus the two lethals are allelic. Hemizygotes for EMS 40-18 and EMS 56-8 are associated with a rather interesting lethal phenotype. These hemizygotes pupate but subsequently undergo complete autolysis with no adult structures present and larval tissues being reduced to an oily mass at the bottom of the pupa case. Thus, the Group IX lethals are associated with a single locus, and when hemizygous exhibit a rather dramatic tissue degradation following pupation.

The analysis of the EMS induced lethal alleles of Df(2L)C' has revealed 7 loci within the deficiency or 3 in addition to the 4 inferred
FIGURE 12

Complementation map of the Group IX lethal alleles of

$\text{Df(2L)C}^\prime$. 
from the compound-second autosome detachment analysis (Chapter II). Multiple lethal alleles were recovered for each locus save one (the site inferred from the existence of the EMS 56-24 lethal) with 2 loci associated with 2 lethal alleles, 3 loci with 3, and 1 locus (the EMS 40-6 complex) with 14 lethal alleles. Cytological observations place all 7 of these loci in the 2L heterochromatin. Df(2L)C' is not deficient for any proximal bands in the euchromatic 2L polytene chromosome nor is it deficient for the secondary constriction at the 2L heterochromatic-euchromatic junction. In addition the C(2R)VK1,Dp(2L)1t bw chromosome, which, as the recovery of the Group F deficiencies demonstrated (see text and Figure 4 of Chapter II), carries a duplication of the 6 loci associated with the Groups VI, VII and VIII lethals, is not duplicated for any portion of the secondary constriction at the 2L heterochromatic-euchromatic junction. Further, although the Group F deficiencies on the basis of their Minute phenotype would appear to have a greater distal extent than the non-Minute Df(2L)C', neither of the 2 Group F deficiencies is deficient for proximal bands in the 2L polytene chromosome and neither of them lacks the 2L secondary constriction. The 4 loci associated with the Groups VIII and IX lethals may lie in the immediate vicinity of the 2L heterochromatic-euchromatic junction as only 1 deficiency deficient for the Group IX lethal locus (the group C' deficiency) and 2 deficiencies deficient for the Group VIII lethal loci (the Groups C and C' deficiencies) were obtained among the C(2L)SH3,+;C(2R)SH3,+ detachments. However, 6 detachments (the Group D deficiencies) deficient for the two loci associated with the Group VII lethals were recovered among the C(2L)SH3,+;C(2R)SH3,+ detachments indicating that these loci and the
more proximal locus associated with the Group VI lethals, for which 14 C(2R)SH3+;C(2R)SH3,+ detachments were deficient (the Groups D and D' deficiencies) are well within the 2L proximal heterochromatin.

Consistent with the findings for EMS induced lethal alleles of Df(2R)MS-10, none of the lethal alleles of Df(2L)C' induced recessive lethals associated with the proximal region of chromosome-2 appear to be deficiencies, it seems evident that, when one employs the mutagenesis procedure of Lewis and Bacher (1968), EMS behaves as a point mutagen in Drosophila heterochromatin, as has previously been demonstrated in Drosophila euchromatin (Lim and Snyder 1974).
CHAPTER IV

GENERAL DISCUSSION
The preceding sections of this thesis describe the genetic dissection of the proximal region of chromosome-2 of Drosophila melanogaster through the detachment of compound-second autosomes (Chapter II) and the induction, with ethyl methane sulphonate (EMS), of recessive lethals allelic to the 2L and 2R proximal deficiencies associated with the detachment products. In combination with cytological analyses of the compound autosomes employed and proximal deficiencies obtained, these studies have demonstrated that genetic loci are situated within the constitutive heterochromatin of the second chromosome. Further, these loci are not associated with secondary constrictions as observed for the bobbed locus in the X-chromosome heterochromatin and, probably, for the fertility factors in the Y-chromosome. These heterochromatic loci appear to be nonrepetitive genes, i.e. only one copy of the structural gene is present at each locus. Lethal alleles of the chromosome-2 heterochromatic loci uncovered in this study have late larval and pupal lethal phases, an observation of possible significance. Finally, although genes have been demonstrated in chromosome-2 proximal heterochromatin, the gene density of this region is very low relative to that estimated for the second chromosome euchromatin (approximately 1%).

The observation of a low gene density in chromosome-2 heterochromatin is perfectly consistent with results previously cited, that have heretofore been interpreted as conclusive evidence of the complete genetic inactivity of heterochromatin (e.g. Yunis and Yasmineh 1971). The assignment of a few genetic loci to a heterochromatic chromosome segment is not of course inconsistent with the assignment of the bulk of the DNA of the heterochromatic segment to short, highly repetitive base sequences. Further
the lack of DNA-RNA hybridization of chromocentral DNA with nuclear (and cytoplasmic) RNA merely demonstrates the apparent genetic inactivity of heterochromatin relative to euchromatin. A few gene loci within a large heterochromatic chromosome segment can not be demonstrated bio-chemically, only genetically, as has been accomplished here for the Drosophila second chromosome heterochromatin. While one may find it difficult to conceive how transcription of RNA may occur at sites intercalated in the condensed chromocentral material, it is evident that DNA replication, a process fundamentally similar to RNA transcription, does occur within heterochromatin. Moreover, it is conceivable that the folding of the heterochromatic material to form the chromocenter may have a pattern of organization such that functional genetic loci intercalated in heterochromatin are positioned on the exterior of the chromocenter. (The significance of the location of those loci intercalated in the chromosome-2 heterochromatin and those associated with heterochromatic-euchromatic junctions is unclear, although the observation that the lethal phases of the lethal alleles of these loci are late larval and pupal is, perhaps, instructive.)

The analyses of the EMS-induced lethal alleles of 2L and 2R proximal deficiencies have demonstrated that EMS behaves as a point mutagen, i.e. EMS does not induce deletions. Lim and Snyder (1974) have arrived at the same conclusion from their analysis of EMS-induced recessive lethals falling within a small segment of X-chromosome euchromatin. Thus, EMS appears to be a nonradiomimetic mutagen in that it does not induce deletions (Chapter III; Lim and Snyder 1974) or other interchanges (Lim and Snyder 1968; W. Gibson unpublished) in Drosophila
heterochromatin or euchromatin. EMS may well operate, therefore, by a base substitution mechanism resulting in the alteration of a single nucleotide in the nucleotide sequence of a gene.

In addition to confirming that EMS does not generate chromosomal aberrations or interchanges, the analysis of the EMS induced chromosome-2 proximal lethals has provided a caveat for the general procedure of defining the genetic constitution of a chromosome segment through the analysis of mutagen induced recessive lethals. This caveat is that the phenomenon of interallelic complementation may lead one to overestimate the number of genetic loci within a chromosome segment under investigation. This possibility is illustrated by the complementation maps of lethal alleles of loci within Df(2R)M-S210. For example, if any one lethal was missing from the EMS 45-10 complex of the Group I lethals (Figure 2 of Chapter III), then the complementation map would resemble that of three overlapping deficiencies. This would imply that at least three genetic loci were associated with the EMS 45-10 complex. Another example is that of the Group V lethals (Figure 7 of Chapter III). Were it not for the existence of EMS 34-4, the complementation of EMS 45-89 with the remaining Group V lethals would suggest that it represented a lethal allele of a second locus. Only when adjacent complementing recessive lethals are separable by a definite deficiency can it be said to be conclusively demonstrated that they represent lethal alleles of separate loci. Furthermore, the results of this study imply that, in Drosophila, when the complementation map of adjacent EMS-induced recessive lethals resembles that of overlapping deficiencies one is, in reality, observing interallelic complementation
among lethal alleles of a single locus.

In addition to the genetic analysis of the proximal heterochromatin in chromosome-2, the effects of various heterochromatic deficiencies and duplications on meiotic segregation, chromosome loss, and recombination were assayed. Heterozygotes for \( \text{Df}(2\text{M-S}\text{2}^10) \), a deficiency for the 2R heterochromatic block, were tested for elevated rates of chromosome-2 nondisjunction and chromosome loss (Appendix V). Virtually no spontaneous nondisjunction or chromosome loss for the second chromosome were observed in males or females heterozygous for the \( \text{M}(2\text{S}\text{10}) \) deficiency. Thus the removal of the proximal heterochromatin to the right of the centromere of chromosome-2 does not result in a meiotically unstable chromosome. In preliminary experiments (Hilliker, unpublished), however, it was found that \( \text{Df}(2\text{R-M-S}\text{2}^10) \) heterozygosity results in a marked reduction in recombination for the right arm of chromosome-2. The nature and significance of this reduction awaits further experimental analysis.

The Group C' deficiency was assayed for heterozygous nondisjunction and chromosome loss. \( \text{Df}(2\text{L-C})' \), in addition to being deficient for much of the 2L proximal heterochromatin, is, on the basis of genetic and cytological evidence, duplicated for a large segment of the 2R heterochromatic block. Heterozygosity for \( \text{Df}(2\text{L-C})' \) is not, however, associated with nondisjunction (or chromosome loss) of the second chromosome (Appendix V).

Further, compound-second autosomes bearing duplications of the proximal heterochromatin of the other chromosome arm were assayed in males for increased meiotic segregation from the complementary compound. In his analysis of the meiotic segregation of several combinations of
compound-second autosomes in males, Holm (1969) demonstrated an apparent random assortment of C(2L) and C(2R), with nearly equal frequencies of C(2L); C(2R); nullo-2; and diplo-2 sperm being produced. In my analysis of the meiotic segregation, in males, of compound autosomes bearing duplications of proximal heterochromatin of the opposite arm I witnessed no increase in the recovery of segregant classes (C(2L); and C(2R) sperm), indicating that these heterochromatic duplications had no effect on the segregation of compound autosomes (Appendix VI).

Finally, this study has provided material for examining the nature of recombination within heterochromatin. Lethal alleles have been generated for loci intercalated in the chromosome-2 heterochromatin; recombinants between these lethals can be easily detected and genetically confirmed; and only large-scale recombination experiments remain to determine whether or not recombination, and/or the related phenomenon of gene conversion (Chovnick, Ballantyne and Holm 1971), occurs within Drosophila heterochromatin.

In conclusion, while it is clear from this study that heterochromatin is not genetically inert, it is evident that gene density is very low relative to euchromatin. Perhaps, as an analysis of the phenomenon of ltr-variegation suggests (Appendix I), the genetically inactive regions serve to provide a necessary environment for the normal expression of genes intercalated in or adjacent to heterochromatic regions. However, although heterochromatin is not completely devoid of genes, the principal functions of heterochromatin, if any, remain undefined.


Sederoff, R., L. Lowenstein and H. C. Birnboim, 1975 Polypyrimidine segments in Drosophila melanogaster DNA: II chromosome location and nucleotide sequence. (submitted for publication.)


APPENDIX I

ON THE NATURE OF POSITION-EFFECT VARIEGATION OF THE LIGHT LOCUS
The light locus of *Drosophila melanogaster* is located near the heterochromatic-euchromatic junction of the left arm of chromosome-2 (Schultz 1936; Chapter II). Position-effect variegation of this locus was first investigated by Schultz and his collaborators (Schultz and Dobzhansky 1934; Morgan, Bridges and Schultz 1932, 1935; Morgan, Schultz and Curry 1941; Morgan and Schultz 1942; Schultz 1936, 1941). Light variegation was readily induced by X-rays and was found to be associated with chromosomal rearrangements involving one breakpoint in the proximal region of 2L and the other in the euchromatin of the X, second, or third chromosome.

Hessler (1958) using X-radiation induced a number of light-variegating chromosomal rearrangements and examined the breakpoints in the salivary gland and somatic chromosomes. From this cytological analysis it was clear that in most light-variegating chromosomal rearrangements one breakpoint was in the distal euchromatin of the X-chromosome or an autosome, and the second breakpoint proximal to the 2L heterochromatic-euchromatic junction.

Hessler interpreted these data to imply that the light locus was located in or next to the proximal heterochromatin of 2L and that the removal of this locus from heterochromatin to distal euchromatin, i.e. euchromatin far removed from a centromere, resulted in variegation.

However, as Hessler recognized, it could not be determined whether the light locus was actually displaced. The breaks may have been distal to the light locus, the juxtaposition of previously distal euchromatin with the basally located light locus resulting in variegation.

The fortuitous construction of a 2;3 translocation from a compound
second autosome bearing female enabled me to obtain a light-variegating rearrangement in which it could be conclusively demonstrated that the light locus was transposed (i.e. distal to the 2L breakpoint). This 2;3 translocation was constructed by the irradiation of C(2L)SH3,+;C(2R)SH3,+ females and is referred to as the Group E lethal in Chapter II. As described in Chapter II, this lethal arose from a 3 hit event that translocated 2L, including a large block of heterochromatin proximal to the 2L secondary constriction to the right arm of chromosome-3 at the 92E-F region of Bridges' polytene chromosome map (see Lindsley and Grell 1968). The right arm of chromosome-3 distal to 92E-F is appended to the centromere-bearing detachment of C(2R) (the break in C(2R)SH3,+ was in the proximal heterochromatin). The centric 2L and acentric 2R fragments, which together represent the reciprocal product of the translocation were lost. Since C(2R)SH3,+ does not carry a lt+ duplication, one may follow with certainty the lt locus, as the translocation derived its 2L from C(2L)SH3,+ , the centromere-bearing fragment of which was lost during the construction of this 2;3 quasi-reciprocal translocation.

The Group E translocation proved to be very weakly homozygous viable and progeny homozygous for the translocation or heterozygous for the translocation and lt had a light-variegated phenotype. In addition, the Group E translocation was lethal in combination with the 2 Group C lt deficiencies (see Chapter II). Further, upon careful examination of the somatic chromosomes it was found that virtually the whole of 2L including the 2L heterochromatic block was translocated to the distal euchromatin of 3R. From the above observations it is clear that it is
the displacement of the light locus to distal euchromatin, rather than the
displacement of previously distal euchromatin into a position in the
vicinity of the light locus, that resulted in light variegation.

However, it would appear that it is not the removal of light
from heterochromatin and its juxtaposition with euchromatin that is
responsible for light variegation, but rather the removal, from the
centromere of the heterochromatic block with which \textit{lt} is associated.
That is, in order for the light locus to function normally, the hetero-
chromatic block with which it is associated must be near a centromere.
Thus, although the heterochromatin immediately adjacent to the \textit{lt} locus
may remain undisturbed by a particular chromosomal rearrangement, light
variegation will result if the 2L heterochromatic block has been
displaced far from a centromere.

Recent studies indicate that the centromeric heterochromatin of
eukaryotes is bound during interphase to the nuclear membrane (see
Harrisson 1971). Thus genes associated with centromeric heterochromatin
may have a somewhat defined environment. It may be that the centromere
migrates to the nuclear membrane when the nuclear membrane reforms
subsequent to the initiation of telophase. Heterochromatin associated
with the centromere may then bind to the nuclear membrane. However, if
by a chromosomal rearrangement, heterochromatin is moved some distance
from the centromere, the probability of binding to the nuclear membrane
may be reduced. That is, the further heterochromatin is removed from
the centromere, the less the probability of its binding to the nuclear
membrane. It is postulated that the association of the 2L heterochromatic
block with the nuclear membrane is necessary for the normal function of
the light locus and that the inhibition of this association by translocating 1t and 2L heterochromatin to distal euchromatin results in light variegation.

In conclusion, light variegation results from the displacement of the light locus and its associated heterochromatic block to a position well removed from a centromere. It is speculated that the physiological basis of light variegation may lie in the effect that the binding of constitutive heterochromatin to the nuclear membrane has on the functioning of individual heterochromatic loci.
APPENDIX II

AN IMPROVED TECHNIQUE FOR
POLYTENE CHROMOSOME SQUASH
PREPARATIONS IN DROSOPHILA
INTRODUCTION

In the past, obtaining consistently good polytene chromosome preparations from the larval salivary glands of *Drosophila melanogaster* has required optimal conditions—fully mature larvae (prepupae) raised in uncrowded cultures at low temperature (Demerec and Kaufmann 1969). By a single mechanical modification of the standard squash technique I have been able to obtain routinely superior preparations under less than optimal conditions. Moreover, this modification allows one easily to obtain good preparations of complex chromosomal rearrangements, heretofore rather difficult to achieve even under optimal conditions.

MATERIALS AND METHODS

1. Dissect third instar Drosophila larvae in a large drop of 50% acetic acid on a depression slide. (Dissection in 45% acetic acid was suggested by T. Kaufman. One may also dissect in Drosophila Ringers' but dilute acetic acid gives better preparations.)

2. Transfer the salivary glands to a drop of 2% aceto-lacto-orcein (Vosa 1961) on a siliconized slide.

3. Place the coverslip on the drop of stain in which the salivary glands are immersed. Use 18 or 22 mm$^2$ coverslips.

4. Tap the coverslip gently 10 or more times directly over the glands with the blunt end of a pair of forceps. This is the innovative step and the most important in the procedure. Although one taps gently, the glands spread out considerably. The exact strength of the taps will be best learned by experience. The coverslip moves laterally somewhat but this movement should be minimal.
5. Squash the glands with the thumb, applying firm but not excessive pressure through paper towelling directly on the coverslip and over the glands. Do not move the coverslip laterally while squashing. Seal the preparation with clear nail polish or wax.

6. Observe with phase contrast optics.

RESULTS

Well spread polytene chromosomes are obtained routinely under less than optimal conditions. One may virtually disregard temperature, and overcrowding and precise larval age are of less concern. One can rely on several good spreads from each preparation, with minimal chromosome breakage and distortion.

With complex chromosome rearrangements the results are particularly pleasing. Normally, even under optimal conditions, good polytene chromosome spreads of such rearrangements are difficult to obtain. One may resort to extreme methods, such as squashing with excessive force or pressing down on the coverslip directly over the salivary glands with the eraser end of a pencil, but these techniques are inefficient and often result in lateral movement of the coverslip and broken or distorted chromosomes that usually are not well spread.

By the new method reported herein, however, one may easily obtain good spreads of complex rearrangements without chromosome breakage or distortion.
DISCUSSION

In the unmodified squash technique nuclei were ruptured and chromosomes were spread and fixed between the slide and coverslip simultaneously. The taps probably shock the nuclei and chromosomes, bursting the nuclei and forcing the chromosomes to disperse, giving them time to do so before fixing them between the coverslip and slide. Once the chromosomes are fixed between the coverslip and slide, additional squashing is relatively ineffective in promoting further chromosome dispersal.
APPENDIX III

PREPARATION OF SOMATIC MITOSES OF DROSOPHILA AND CHROMOSOME IDENTIFICATION
Chromosome preparation (modified from Moore 1971)

1. Select an active mature third-instar larvae and dissect out the brain in Drosophila Ringers' (0.75% NaCl).
2. Place the brain in 1% Na Citrate for 30 to 90 seconds.
3. Place the tissue in a drop of 2% aceto-lacto-orcein (Vosa 1961) on a siliconized slide and allow it to fix and stain for 5 minutes at room temperature. Prefixation in any of acetic-methanol (various proportions); and Farmer's; Navashin's; Carnoy's; Flemming's; and Benda's fixatives (Sharma and Sharma 1972), I found to be of little value save for Benda's fixative if one desires to observe anaphase chromosomes.
4. Drop a coverslip onto the drop of stain.
5. Squash with the thumb, applying firm pressure through paper towelling directly on the coverslip and over the tissue. Do not move the coverslip laterally while squashing.
6. Seal the edges of the coverslip with clear nail polish or wax.
7. Observe the preparation 24 to 48 hours later with phase-contrast optics.
Chromosome identification

This account is based largely on my own observations. Valuable published descriptions of *Drosophila melanogaster* somatic chromosome morphology include Kaufmann (1934) and Cooper (1959).

*Drosophila melanogaster* has four pairs of chromosomes, one pair of which is heteromorphic in the male (the X/Y bivalent). The X-chromosome is acrocentric; the Y-chromosome, submetacentric; the second and third chromosomes are metacentric; and the tiny fourth chromosome is probably acrocentric.

The proximal third of the X-chromosome is heterochromatic. This heterochromatic region is often subdivided into two equal blocks by a prominent secondary constriction, viz. the NO (nucleolar organizer). Much less frequently, other, smaller constrictions are observed which further subdivide the X-chromosome heterochromatin (see Cooper 1959).

The Y-chromosome is completely heterochromatic. A NO is present on the short arm (Y₅). Six other Y-chromosome secondary constrictions have been observed (Cooper 1959); two of which, on the long arm (Y₇), I have frequently observed.

The major autosomes, chromosomes 2 and 3, are of roughly equal size, the third chromosome being somewhat longer. In overall length the major autosomes are roughly twice the size of the X-chromosome. The centromeres of these chromosomes are bounded on each side by heterochromatin. The proximal quarter (roughly) of each autosomal arm is heterochromatic. In favorable preparations it can be observed that while the 3L and 3R heterochromatic blocks are of approximately equal mass, the 2R hetero-
chromatic block is somewhat larger than that of 2L. Further, the heterochromatic-euchromatic junction of 2L is the site of a very frequently observed secondary constriction (Kaufmann 1934). This 2L secondary constriction is as prominent as that associated with the X-chromosome and may be considered a very important landmark. Much less frequently, secondary constrictions are observed at the heterochromatic-euchromatic junctions of the other autosomal arms, although never at the heterochromatic-euchromatic junction of the X-chromosome.

I have observed in early prophase nuclei indications of secondary constrictions within the autosomal heterochromatic blocks. However, these constrictions are not prominent and are not observed at late prophase.

The distribution of heterochromatin on the small fourth chromosome is highly debatable.

Constrictions are also observed occasionally within the euchromatin; although this is controversial. Often I have observed what appears to be a constriction in the distal third of the X-chromosome euchromatin.

The left and right arms of chromosome three can be differentiated easily only during early prophase, in which the greater length of 3R is, in favourable preparations, clearly observable.

Chromosome morphology is more distinct in early than in late prophase nuclei. In early prophase nuclei the chromatids remain in apposition, that is each chromosome is not separated into two chromatids. The heterochromatic blocks are easily separable from euchromatin on the basis of their much higher degree of condensation. It is in early prophase nuclei that secondary constrictions may be most profitably studied.
However, given the lack of condensation of the euchromatic regions, the chromosomes are less distinct, longer, and overlap each other considerably.

By late prophase the euchromatic portions of the chromosomes have separated into chromatids. The heterochromatic regions, however, remain together and this property of chromatid apposition is characteristic, in fact diagnostic, of heterochromatin in late prophase. Although the heterochromatic regions may be resolved into chromatids precociously (usually by energetic squashing!), ordinarily they do not separate into chromatids until the onset of anaphase. By late prophase the euchromatin has considerably condensed and the differential staining intensity between it and heterochromatin is much less marked. Prominent secondary constrictions are less pronounced and smaller constrictions usually are no longer distinguishable. Late prophase chromosomes are more easily separated by squashing than those of early prophase and are more distinct.

For routine karyotype analysis late prophase figures are most suitable. However, for detailed morphological analysis early prophase nuclei must be studied.
APPENDIX IV

THE LOCATION OF THE ROUGHISH LOCUS
The roughish (rh) locus, the mutant recessive allele of which is associated with a rough eyed phenotype, is reported to map at 54.7 on the second chromosome of *Drosophila melanogaster* (Lindsley and Grell 1968). This places rh in the proximal region of 2L, distal to lt, tri, 1(2) crc, esc, and Bl. (For a description of these and other genetic markers discussed in this section see Table I of Chapter II.) As rh is a proximal gene, the rh mutant allele was tested against the proximal deficiencies described in Chapter II for pseudodominance in order to ascertain whether the rh locus fell within the heterochromatic regions defined by these deficiencies. However, none of the proximal deficiencies uncovered the rh locus.

Coincident with the testing of chromosome-^proximal^ mutations with proximal deficiencies, attempts were made to ascertain the accuracy of assignment of these loci to chromosome arms. Deciding on which side of the centromere a proximal locus lies is difficult to accomplish by conventional genetic mapping.

A superior method of determining on which arm of an autosome a proximal locus is situated is to construct compound autosomes for the chromosome in question from strains homozygous for a mutant allele of the proximal gene (Holm, Baldwin, Duck and Chovnick 1969). If the gene's mutant phenotype is associated with the newly induced compound left autosome, then the locus is to the left of the centromere. Similarly, if the mutant phenotype is associated with newly induced compound right autosomes, then the gene is in the right arm. By this method D. Holm has confirmed the assignment of lt and H. Harger, the assignment of Bl to 2L; and D. Holm has confirmed the assignment of rl and pk, J. Gavin, the assignment of stw, W. Gibson the assignment of ap, and A Hilliker, the assignment
of M(2)S2, tuf, and ltd to 2R.

However, I found that rh, which had been tentatively assigned to 2L (Lindsley and Grell 1968) was, in fact, actually located on 2R. From the irradiation (4250 rads) of 355 rh/rh virgin females which were subsequently single pair mated to C(2L)P,b C(2R)VHk1,rlcn males and brooded for 7 days, 44 exceptional F1 progeny were obtained (Table I). Of these exceptional progeny, 2 were matroclinous; 33, patroclinous; and 9 represented newly induced compound autosomes. The rh phenotype was associated with all newly induced C(2R) but none of the 5 newly induced C(2L) chromosomes, thereby demonstrating that rh is on the right arm of chromosome two and not the left as previously reported. (Note that all 5 of the newly induced C(2L)'s gave a rl+ phenotype in combination with C(2R)VHk1,rlcn. Many C(2L) chromosomes, synthesized from a variety of strains, have been found (by W. Gibson, T. Yeomans, D. Holm, and myself) to carry rl+ duplications of 2R.)
TABLE I

Exceptional progeny recovered from irradiated \( rh/rh \) virgin females crossed to \( C(2L)P,b \) \( C(2R)VHKl,r\) \( lcn \) males.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Number recovered</th>
<th>Phenomenon</th>
</tr>
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<tbody>
<tr>
<td>( rh ) (matroclinous)</td>
<td>2</td>
<td>Nondisjunction</td>
</tr>
<tr>
<td>( b; rl; on ) (patroclinous)</td>
<td>33</td>
<td>Nondisjunction or Chromosome loss</td>
</tr>
<tr>
<td>( on )</td>
<td>5</td>
<td>Newly induced ( C(2L) )</td>
</tr>
<tr>
<td>( b; rh )</td>
<td>4</td>
<td>Newly induced ( C(2R) )</td>
</tr>
</tbody>
</table>
APPENDIX V

PRELIMINARY EXPERIMENTS ON
THE EFFECTS OF HETEROZYGOSITY
FOR SECOND CHROMOSOME PROXIMAL
DEFICIENCIES ON MEIOSIS
It has been suggested (Walker 1971) that centromeric heterochromatin may promote the initiation of meiotic pairing of homologous chromosomes and, further, protect the centromere from the "rigours of meiosis" - presumably the terminalisation of chiasmata and subsequent reductional segregation of homologous dyads. This hypothesis suggested the following experiments, namely assaying the effects of chromosome-2 proximal deficiencies on second chromosome nondisjunction and loss. Walker's theory (loc. cit.) would suggest that heterozygosity for second chromosome heterochromatic deficiencies will result in appreciable second chromosome nondisjunction and chromosome loss. The proximal deficiencies studied were Df(2R)M-S210, which, as previously described (Chapter II), is deficient for the 2R heterochromatic block and Df(2L)C', which is undoubtedly deficient for much of the 2L proximal heterochromatin (Chapter II), although cytological observations imply that it must therefore be duplicated for much of the 2R heterochromatin.

**Df(2R)MS-10:**

Virgin females heterozygous for Df(2R)M-S210 and b pr cn (for a description of these mutations see Table I of Chapter II) were crossed, singly in vials, to C(2L)VHI,1t; C(2R)P,px males and brooded for six days. As compound-second autosome bearing males produce nearly equal frequencies of the four classes of C(2L); C(2R); nullo-2; and diplo-2 sperm (Holm 1969), nullo-2 and diplo-2 female gametes, the consequence of second chromosome nondisjunction or chromosome loss, may be recovered as viable zygotes with 25% efficiency. Thus by the use of multipliers to estimate the total number of fertilized eggs (see MATERIALS AND METHODS)
section of Chapter II), one may assay second chromosome loss and nondisjunction in Drosophila females by mating them with compound second autosome bearing males.

From an estimated 4844 fertilized eggs, no nondisjunctional progeny were recovered. Thus neither chromosome loss nor nondisjunction of chromosome two is associated with heterozygosity for \text{Df(2R)M-S2}$^{10}$. The absence of chromosome loss is a partial refutation of Walker's (loc. cit.) theory of the protection of centromeres by flanking heterochromatin. No newly induced isochromosome bearing exceptions (a possible consequence of chromosome "breakage") or patroclinous progeny were observed. Clearly the \text{M(2)S10} chromosome is stable despite the absence of the 2R heterochromatic block. The upper 95% confidence limit of second chromosome loss, and therefore chromosome instability is 0.25%. (Nor did \text{M(2)S10/b pr cn} heterozygotes prove particularly sensitive to radiation-induced nondisjunction. From mating \text{Df(2R)M-S2}$^{10}$/b pr cn females irradiated with 2000 rads of gamma radiation to \text{C(2L)VH1,lt;C(2R)P,px} males only 1 matroclinous progeny was observed in an estimated 44,269 fertilized eggs, although 33 patroclinous progeny and 15 newly induced compound second autosome bearing exceptional progeny were recovered.)

Second chromosome nondisjunction, and loss, was also assayed in males heterozygous for \text{Df(2R)M-S2}$^{10}$ and \text{In(2LR)SML}. \text{Df(2R)M-S2}$^{10}$/\text{In(2LR)SML} males were crossed to \text{B^S}Y;\text{C(2L)P,b;C(2R)P,px} virgin females, singly in vials and brooded for 9 days. \text{B^S}Y is a \text{Y}-chromosome carrying a small duplication of the \text{X}-chromosome including a dominant allele of the Bar (\text{B}) locus (see Lindsley and Grell 1968 for further details). \text{B^S}Y;\text{C(2L)P,b;C(2R)P,px} females produce 40% second chromosome non-
segregational progeny when crossed to compound second autosome bearing males. Thus 20% of the female gametes are diplo-2 and 20% are nullo-2 (see Appendix VI). Consequently, nondisjunction for chromosome 2 may be assayed in males by mating them to $B^y;C(2L)P;b;C(2R)P,px$ females.

In order to estimate the number of nondisjunctional female gametes produced per female per vial, a sample of $B^y;C(2L)P,b;C(2R)P,px$ virgin females were crossed to $C(2L)SH3,+;C(2R)SH3,+ \text{ males}$. As these males produce approximately 25% diplo-2 and 25% nullo-2 sperm, the nondisjunctional progeny represent 1/4 of the nondisjunctional female gametes. Thus, the number of nondisjunctional female gametes per experimental vial may be estimated as 4 times the number of nondisjunctional progeny per multiplier vial. However, in the cross to the $Df(2R)M-S2^{10}/In(2LR)SM1$ males a nondisjunctional (diplo-2 or nullo-2) sperm fertilizing a nondisjunctional female gamete has a 50% chance of resulting in a viable, diplo-2 zygote.

Thus chromosome-2 nondisjunction per experimental vial may be estimated as 2 times the number of exceptional (i.e. nondisjunctional) progeny per experimental vial divided by 4 times the number of nondisjunctional progeny per multiplier vial. In the experimental series, an estimated 8810 nondisjunctional female gametes resulted in no diploid nondisjunctional progeny. (The only exceptional progeny recovered were one triploid female and 2 intersexes.) Thus a 95% upper confidence limit on chromosome-2 nondisjunction in $Df(2R)M-S2^{10}/In(2LR)SM1$ heterozygotes of 0.068% is established. With chromosome-2 nondisjunction and chromosome loss less than 0.1% in males and 0.3% in females heterozygous for $Df(2R)MS-10$, the loss of the heterochromatic block to
the right of the centromere of chromosome-2 clearly does not result in any meiotic instability of the second chromosome.

**Df(2L)C'**

Virgin females heterozygous for Df(2L)C' and b pr cn were mated to C(2L)VH1,lt;C(2R)P,px males and brooded for 6 days. In an estimated 31,200 fertilized eggs, 2 matroclinous and 1 patroclinous progeny were recovered. The frequency of spontaneous second chromosome nondisjunction in Df(2L)C' heterozygotes is 4x3/31,200 or 0.038% with 95% confidence limits of 0.008% and 0.112% (Stevens 1942), well within the range observed for Drosophila females homozygous for normal second chromosomes (W. Gibson, personal communication).

Although the genetic evidence (Chapters II and III) strongly argued that Df(2L)C' was deficient for much of the 2L heterochromatin, upon cytological examination of the Df(2L)C' somatic chromosome I found a substantial block of heterochromatin to the left of the centromere. This could be explained by the following hypothesis. In the construction of Df(2L)C' from the detachment of C(2L)SH3,+;C(2R)SH3,+ the acentric 2L fragment was generated by a break in the distal 2L heterochromatin (that this break was proximal to the secondary constriction at the 2L heterochromatic-euchromatic junction was clear, as Df(2L)C' was not deficient for this constriction) with the centric 2R fragment being generated by a break in the distal heterochromatin of 2R resulting in a centric 2R fragment duplicated for much of the 2R heterochromatin including the r1+ locus. Df(2L)C' therefore would be a 2L proximal deficiency but a 2R proximal duplication with a r1+ locus on each side
of the centromere.

In order to test the hypothesis I constructed with radiation non-
sister 2L compound autosomes (compound autosomes with one 2L chromatid
from one second chromosome and the other 2L chromatid from its
homologue) from females heterozygous for Df(2L)C' and b pr cn.
If Df(2L)C' carries a rl+ duplication in the left arm then compound
left autosomes deriving one arm from the Df(2L)C' chromosome should
more frequently carry rl+ duplications of 2R than do compound lefts
derived from normal second chromosomes. Of 21 nonsister compound left
autosomes derived from Df(2L)C'/b pr cn heterozygotes, 17 were rl+
whereas Yeomans (1972) found only 10 of 21 compound left second chromosomes
derived from 1t stw3/b pr cn heterozygous females were rl+ and W. Gibson
(unpublished), upon examining 39 compound left second autosomes derived
from females of a number of different heterozygous genotypes, found
that 25 carried a duplication rl+. Thus Df(2L)C' would appear to be
duplicated for rl+ and, therefore, much of the 2R heterochromatin.
This conclusion is also supported by biochemical evidence. Sederof et
al. (1975) have found that the highly repeated polypyrimidine sequence
TCTTC is localized to the Y-chromosome and the 2R heterochromatin.
J. Stone (personal communication) has found that the nuclear DNA of
Df(2L)C' heterozygotes has a larger fraction of the TCTTC repeated
pentamer than does the nuclear DNA of wild type Drosophila.
APPENDIX VI

HETEROCHROMATIC DUPLICATIONS
AND THE MEIOTIC SEGREGATION
OF COMPOUND AUTOSOMES IN MALE
DROSOPHILA
Holm (1969) demonstrated that in the several strains he examined compound second autosomes segregated randomly during male meiosis producing equal frequencies of $C(2L); C(2R); \text{diplo-2} (C(2L);C(2R))$ and nullo-2 (neither $C(2L)$ nor $C(2R)$) bearing sperm. In female Drosophila, however, Holm found that the $C(2L)$ chromosome regularly segregates from the $C(2R)$ chromosome.

In order to examine the role, if any, of heterochromatic homology in meiotic pairing in males, the segregation of compound autosomes bearing duplications for heterochromatic material of the complementary compound autosomes was assayed. Segregation was assayed by crossing males of the selected compound-second autosome bearing strains to differentially marked compound-second autosome bearing females possessing a Y-chromosome. These $^{S^Y}; C(2L)P,b; C(2R)P,px$ females give, as first demonstrated by E. H. Grell (1970) for $^{S^Y}; C(2L); C(2R)$ bearing females in general, a high frequency of compound-second autosome nonsegregation. Female gametes nonsegregational for the compound second autosomes will result in a viable zygote only if fertilized by a sperm nonsegregational for the paternal compound-second autosomes. Thus a strain in which compound-second autosomes partially segregate in males when crossed to $^{S^Y}; C(2L)P,b; C(2R)P,px$ females will give a lower frequency of progeny completely matroclinous or patroclinous for the two compound-second autosomes than will a strain in which $C(2L)$ and $C(2R)$ segregate at random in the male.

To examine the effect, if any, of heterochromatic homology on compound-second autosome meiotic segregation in males, males of the $C(2L)SH3+;C(2R)SH3+$ strain (a strain in which nearly equal frequencies
of C(2L); C(2R); diplo-2; and nullo-2 sperm are produced) and of several other strains in which one or both compound autosomes bore heterochromatic duplications of the other arm were crossed singly in shell vials to B^S_Y;C(2L)P,b;C(2R)P,px virgin females. The results are presented in Table I and in summary form in Table II.

Since there is no significant reduction in the frequency of non-segregation in those crosses involving males carrying C(2L) and C(2R) chromosomes with measurable heterochromatic homology (Table II), it is apparent that heterochromatic homology is not a major factor in regulating segregation in males of Drosophila melanogaster.
TABLE I

Progeny of $B^S_Y; C(2L)P,b; C(2L)P,b; C(2R)P,px$ females and various compound second autosome bearing males.

<table>
<thead>
<tr>
<th>Male genotype</th>
<th>$B^S_Y$</th>
<th>$B^S_Y$</th>
<th>$B^S_Y$</th>
<th>$B^S_Y$</th>
<th>$B^S_Y$</th>
<th>$B^S_Y$</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C(2L)SH3,+$</td>
<td>1</td>
<td>462</td>
<td>300</td>
<td>273</td>
<td>204</td>
<td>279</td>
<td>247</td>
</tr>
<tr>
<td>$C(2R)SH3,+$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>$C(2L)SH1,Dp(2R)rl^+$</td>
<td>1</td>
<td>567</td>
<td>395</td>
<td>365</td>
<td>286</td>
<td>383</td>
<td>447</td>
</tr>
<tr>
<td>$C(2R)SH1,+$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>$C(2L)SH1,Dp(2R)rl^+$</td>
<td>0</td>
<td>57</td>
<td>39</td>
<td>46</td>
<td>31</td>
<td>47</td>
<td>44</td>
</tr>
<tr>
<td>$C(2R)SH3,+$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>$C(2L)VH3,+$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1771</td>
</tr>
<tr>
<td>$C(2R)VK2,Dp(2L)lt^+,bw$</td>
<td>0</td>
<td>127</td>
<td>153</td>
<td>146</td>
<td>122</td>
<td>174</td>
<td>215</td>
</tr>
<tr>
<td>$C(2L)VH1,Dp(2R)rl^+,lt$</td>
<td>1</td>
<td>122</td>
<td>127</td>
<td>102</td>
<td>80</td>
<td>146</td>
<td>163</td>
</tr>
<tr>
<td>$C(2R)VK2,Dp(2L)lt^+,bw$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
TABLE II.

Frequency of progeny nonsegregational for compound-second autosomes from $B^8 Y; C(2L)P,b; C(2R)P,pX$ virgin females crossed to various strains of compound-second autosome bearing males.

<table>
<thead>
<tr>
<th>$C(2L)$</th>
<th>$C(2R)$</th>
<th>Percent nonsegregational progeny</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>$SH3^+$</td>
<td>$SH3^+$</td>
<td>40.4</td>
<td>1771</td>
</tr>
<tr>
<td>$SH1^+$</td>
<td>$SH1^+$</td>
<td>41.6</td>
<td>2448</td>
</tr>
<tr>
<td>$SH1^+$</td>
<td>$SH3^+$</td>
<td>38.3</td>
<td>264</td>
</tr>
<tr>
<td>$SH3^+$</td>
<td>$VK2bw^{**}$</td>
<td>36.7</td>
<td>940</td>
</tr>
<tr>
<td>$VH1lt^{*}$</td>
<td>$VK2bw^{**}$</td>
<td>38.7</td>
<td>742</td>
</tr>
</tbody>
</table>

* Bears a $rL^+$ duplication of 2R.

** Bears a $Lt^+$ duplication of 2L.