GENETIC ANALYSIS OF THE CENTROMERIC HETEROCHROMATIN OF CHROMOSOME *2* OF *DROSOPHILA MELANOGASTER*: DEFICIENCY MAPPING OF EMS-INDUCED LETHAL COMPLEMENTATION GROUPS.[1,2]

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ABSTRACT

Until recently, little was known of the genetic constitution of the heterochromatic segments of the major autosomes of *Drosophila melanogaster*. Our previous report described the genetic dissection of the proximal, heterochromatic region of chromosome *2* of *Drosophila melanogaster* by means of a series of overlapping deficiencies generated by the detachment of compound second autosomes (HILLIKER and HOLM 1975). Analysis of these deficiencies by *inter se* complementation, pseudo-dominance tests with proximal mutations and allelism tests with known deficiencies provided evidence for the existence of 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 demonstrated that light and rolled and three loci lying between them are located within the proximal heterochromatin of the second chromosome.——The present report describes the further analysis of this region through the induction with ethyl methanesulphonate (EMS) of recessive lethals allelic to the *2L* and *2R* proximal deficiencies associated with the detachment products. 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 junctions of *2L* and *2R* respectively. None of the 113 EMS lethals behaved as a deficiency, implying that the heterochromatic loci uncovered in this study represent nonrepetitive cistrons. Thus functional genetic loci are found in heterochromatin, albeit at a very low density relative to euchromatin.

THE biological function of heterochromatin has been debated since its first clear description by HEITZ (1928, 1929). This debate has become one of broad interest with the demonstration of the ubiquity of heterochromatin among plants and animals (BROWN 1966). An organism which has proven particularly useful

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in the study of the properties of heterochromatin is *Drosophila melanogaster*. The distribution of heterochromatin on the chromosomes of this species was originally described by HEITZ (1933) and KAUFMANN (1934). They found that the entire *Y* chromosome, the proximal ⅓ to ½ of the *X* chromosome, and the proximal ¼ of each of the arms of the major metacentric autosomes, chromosome *2* and *3*, are heterochromatic at prometaphase. These heterochromatic regions are permanently heteropycnotic remaining, unlike the euchromatic regions, as condensed blocks of chromatin throughout the cell cycle. In interphase nuclei these heterochromatic blocks are referred to as chromocentres, which often fuse to form one large composite chromocentre. In early prophase heterochromatic regions are considerably more condensed than the remaining euchromatin. During late prophase heterochromatic regions exhibit the property of chromatid apposition, unlike the euchromatic regions which have separated to reveal two chromatids per chromosome.

In addition to cytological parameters, there are other established differences between heterochromatin and euchromatin in Drosophila. Little recombination occurs in Drosophila heterochromatin (BAKER 1958; SCHALET 1968). Further, cytophotometric data (RUDKIN 1969) confirm the conclusion of HEITZ (1933) that heterochromatin is exceedingly underreplicated during polytene chromosome formation. Drosophila heterochromatin completes DNA replication much later in the S phase of the cell cycle than does euchromatin (BARIGOZZI *et al.* 1966); indeed, the completion of DNA synthesis late in the S phase is characteristic of heterochromatin in all species (LIMA-DE-FARIA and JAWORSKA 1966). Drosophila heterochromatin has the property of inducing the variegated expression of euchromatic loci placed by chromosomal rearrangement immediately adjacent to a heterochromatic segment. (This phenomenon, termed position-effect variegation, is reviewed in LEWIS 1950; HANNAH 1951; and BAKER 1968).

Modern biochemical and cytochemical studies have demonstrated that Drosophila heterochromatin is enriched in highly repetitive DNA (BOTCHAN *et al.* 1971; GALL, COHEN and POLAN 1971; PEACOCK *et al.* 1973). Indeed, in most animal species heterochromatin is enriched in highly repetitive DNA, with the Chinese hamster (*Cricetulus griseus*) being the only known exception (COMINGS and MATTOCCIA 1972; ARRIGHI *et al.* 1974). These highly repeated DNA sequences are often very short in length, of the order of magnitude of 10 nucleotides, and, in all probability, not transcribed (YUNIS and YASMINEH 1971). In *Drosophila melanogaster* there are seven highly repeated DNA sequences which make up the bulk of the constitutive heterochromatin of this species (PEACOCK *et al.* 1973; SEDEROFF, LOWENSTEIN and BIRNBOIM 1975); one of these sequences being an AGAAG pentamer which is localized to the heterochromatin of the second and Y chromosomes.

From their cytogenetic studies on *Drosophila melanogaster* HEITZ and KAUFMANN (*loc. cit.*) facilitated inferences as to the genetic constitution of heterochromatin. With respect to the *Y* chromosome their observations were of considerable significance as the earlier studies of BRIDGES (1916) had demonstrated an apparent lack of genes on the *Y* chromosome. BRIDGES and his colleagues had

discovered no instance of a *Y* linked gene, and, further, BRIDGES found that nullo-*Y* (*XO*) males were of perfectly normal phenotype, viability and behaviour, although sterile. Thus it appeared that the completely heterochromatic *Y* chromosome contained no genes despite the fact that it was larger than the *X* chromosome, for which many genetic loci were known.

However, the description of the *Y* chromosome as completely heterochromatic and genetically inert was to be modified. Several *Y* chromosome secondary constrictions were described (reviewed in COOPER 1959). Further, as BRIDGES' (1916) discovery of the sterility of *XO* males anticipated, specific fertility factors on the *Y* chromosome were subsequently revealed (reviewed in BROSSEAU 1960; 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 may be the sites of the *Y* chromosome genetic loci.

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 had demonstrated that very few if any genetic loci were located in the proximal third of the *X* chromosome, precisely the region of the *X* chromosome 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*), 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.

Until recently, little was known of the genetic constitution of the heterochromatic segments of the major autosomes of *Drosophila melanogaster*. Our previous report described the genetic dissection of the proximal, heterochromatic region of chromosome *2* of *Drosophila melanogaster* by means of a series of overlapping deficiencies generated by the detachment of compound second autosomes (HILLIKER and HOLM 1975). Analysis of these deficiencies by *inter se* complementation, pseudo-dominance tests with proximal mutations and allelism tests with known deficiencies provided evidence for the existence of 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 demonstrated that light and rolled and three loci lying between them are located within the proximal heterochromatin of the second chromosome.

To supplement and further this analysis of the second chromosome proximal heterochromatin, I turned next to the induction of lethal alleles of chromosome *2* proximal deficiencies through the use of the chemical mutagen ethyl methanesulphonate (EMS). In this fashion I could determine if loci in addition to those identified by the detachment analysis existed within the second chromosome heterochromatin and possibly deduce something about the nature of these chromocentral loci.

This paper describes the results of the analysis of EMS-induced lethal alleles of the second chromosome proximal deficiencies *Df(2L)C'* and *Df(2R)M-S2^{10}*. *Df(2L)C'* is the largest *2L* proximal deficiency generated by compound second autosome detachment and *Df(2R)M-2^{10}* is deficient for the *2R* centromeric heterochromatin (HILLIKER and HOLM 1975).

MATERIALS AND METHODS

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

A stock isogenic for chromosome *2* and homozygous for the dominant mutation *Pin* (2:107.3) was derived. *Pin/Pin* (*iso-2*) virgin males aged 2 to 3 days (posteclosion) were fed for 24 hours on a solution of 0.025M 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^{V1}/ In(2LR)SM1,Cy* 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^{10}/In(2LR)SM1,Cy* virgin females. F2 cultures in which all progeny were of the *Cy* phenotype were scored as putative lethal alleles of *Df(2R)M-S2^{10}*. In such cultures the presence of *Pin* on the treated chromosome permitted the derivation of a balanced stock heterozygous for the putative lethal allele of *Df(2R)M-S2^{10}* and *In(2LR)SM1,Cy*.

Each putative lethal allele of *Df(2R)M-S2^{10}* was subsequently tested for complementation with the *2R* proximal deficiencies described in HILLIKER and HOLM (1975). Those putative lethal alleles of *Df(2R)M-S2^{10}* that proved to be lethal alleles of the *M(2)S10* deficiency, rather than lethal alleles of secondary mutations accumulated by the *M(2)S10* chromosome, were tested for complementation in all *inter se* combinations. As all of the proximal lethals and deficiency stocks were heterozygous for *In(2LR)SM1,Cy*, complementation was indicated by the presence of non-*Cy* progeny, whose phenotype was carefully examined. Observations of complementation of key importance in establishing the nature of the often complex allele complementation maps were reconfirmed.

A control experiment was done for the *Df(2R)M-S2^{10}* lethal allele screen.

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

Generation of lethal alleles of Df(2L)Group C':

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

A phenomenon noted in the *Df(2R)M-S2^{10}* 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,Cy*. 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. Such cultures were not numbered among the tested chromosomes in either of the mutagenesis screens.

An assay of the spontaneous mutability of the iso-2 Pin *strain*:

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 these 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.

All mutants and rearrangements not described in LINDSLEY and GRELL (1968) are described in HILLIKER and HOLM (1975).

RESULTS

Lethal alleles of Df(2R)M-S2^{10}:

In the control experiment, no lethal alleles of *Df(2R)M-S2^{10}* were recovered in 1925 tested chromosomes. In the EMS-treated series, 85 lethal alleles of *Df(2R)M-S2^{10}* 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-S2^{10}*, 41 chromosomes were recovered which, although lethal when heterozygous with the *M(2)S10* chromosome, were not lethal alleles of the *M(2)S10* deficiency. Rather, they were alleles of secondary lethals that the *M(2)S10* chromosome had accumulated. That these secondary lethals were not lethal alleles of the *M(2)S10* deficiency was clear, as they complemented with the entire set of *2R* proximal deficiencies which, taken together, encompass the *M(2)S10* deficiency (Figure 1). Of these 41 secondary lethals 8 were mapped and all fell 10 or more crossover units from *rl* and, therefore, well outside the *M(2)S10* deficiency. The accumulation of lethals on permanently heterozygous second chromosomes has been well documented (MUKAI 1964), and the *M(2)S10* chromosome was constructed over 35 years ago (MORGAN, SCHULTZ and CURRY 1940) and continuously maintained in highly inbred cultures since that time.

On the basis of complementation with the 35 available *2R* proximal deficiencies described in our previous report (HILLIKER and HOLM 1975) and the recessive mutation *rl*, the 85 lethal alleles of *Df(2R)M-S2^{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

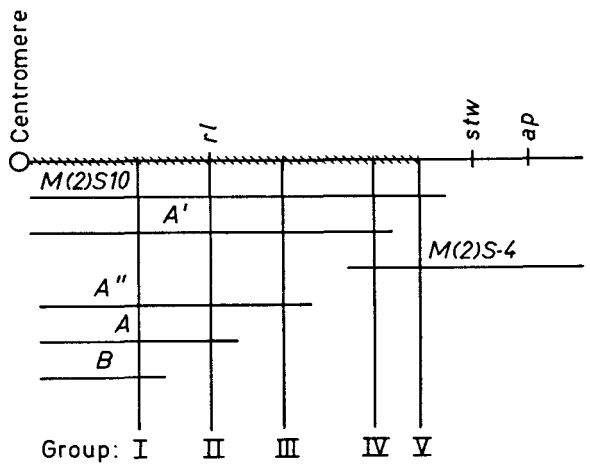

FIGURE 1.—Distribution by complementation with *2R* proximal deficiencies of EMS-induced lethal alleles of *Df(2R)M-S2^{10}*. Proximal heterochromatin is indicated by hatching.

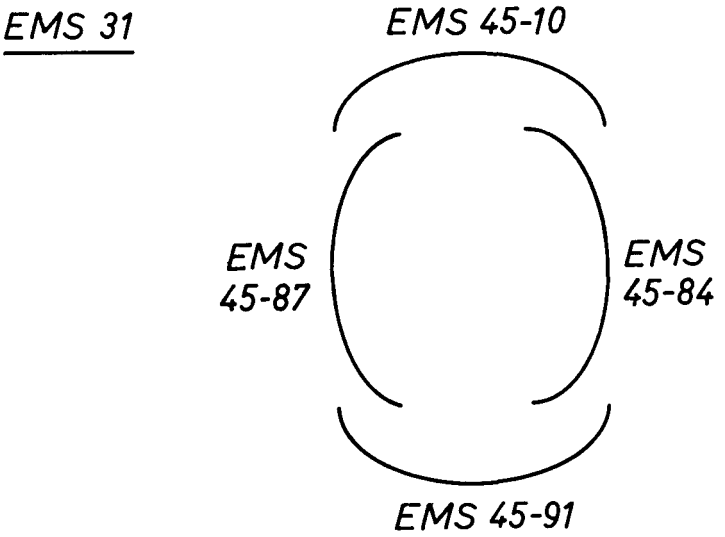

FIGURE 2.—Complementation map of the Group I lethal alleles of *Df(2R)M-S2*^{10}.

B, A, A′ and A″ deficiencies (Figure 1), but they were *rl*$^+$ and survived when heterozygous with *Df(2R)M-S2*^{4}. The complementation map of the Group I lethals is presented in Figure 2.

The Group I lethals may be divided 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). Observations on the phenotype of Group B deficiency homozygotes, and heterozygotes with the Group I lethals and the Groups A, A′, and A″ and *M(2)S10* deficiencies, strongly imply that *EMS 31* represents a locus separate from the one associated with hte *EMS 45–10* complex. Zygotes homozygous for the Group B deficiency and any larger *2R* deficiency encompassing 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. Heterozygotes for *EMS 31* and any of the Groups A, A′, A″ and B or *M(2)S10* deficiencies have the Group B deficiency homozygote lethal 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 when hemizygous. Thus, it would appear that the Group I lethals represent two loci, one, corresponding to the previously inferred *1(2R)B* (HILLIKER and HOLM 1975), 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, preliminary experiments (HILLIKER 1975 and unpublished) demonstrate that it will be difficult, if not impossible, to separate these two sites through recombination.

The Group II lethals (Figure 1) are all alleles of the *rl* locus. 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 deficiency as well as with the *M(2)S4* deficiency and the Groups I, III, IV and V 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. The remaining Group II lethal, *EMS 45–52*, while extremely inviable when homozygous, when heterozygous with *rl* is fully viable and exhibits a *rl* phenotype. Of some interest is the observation that the Group II lethals (excepting *EMS 45–52*) die as third instar larvae that completely lack imaginal discs. To insure that this phenotype reflects a specific Group II lethal effect, all of these observations were made in hemizygous individuals. In contrast, mutant heterozygotes with *EMS 45–52* or *rl* are pupal lethals.

The Group III lethals (Figure 1) subdivide the previously described Group A deficiencies (HILLIKER and HOLM 1975) into two classes, Group A deficiencies that do not include the Group III lethals, and Group A″ that do. The 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 *inter se* combination and thus would definitely appear to be associated with a single locus. Of 33 deficiencies that uncovered *rl* (Groups A, A′ and A″ of Figure 1), 21 include this locus.

Results from compound-autosome detachment studies indicate that all breaks in *C(2R)* associated with the generation of detachments occurred proximal to the heterochromatic-euchromatic junction (HILLIKER and HOLM 1975). Since approximately one-half of the *2R* heterochromatic deficiencies included the marker *rl*, the physical location of this locus was estimated to be near the middle of the *2R* heterochromatic block. Following this same line of argument, the inclusion of the Group III locus in slightly more than one-half of the *rl* deficiencies would place it just proximal to the distal border of the penultimate quarter of the *2R* heterochromatic segment.

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 third legs, etched tergites and smaller body size. Thus, on the basis of phenotype, this site may correspond to the previously described *uex* (unextended) locus (MAEDA 1962), the single mutant allele of which has apparently been lost (LINDSLEY and GRELL 1968).

The next group, the Group IV lethals (Figure 1), presents a rather complex complementation map (Figure 3). In addition to *Df(2R)M-S2*^{10}, the Group IV lethals are lethal in combination with *Df(2R)M-S2*^{4} and the single Group A′ deficiency. As the Group IV lethals fall within the *M(2)S4* deficiency (a deficiency for which I can detect neither loss of *2R* proximal heterochromatin in

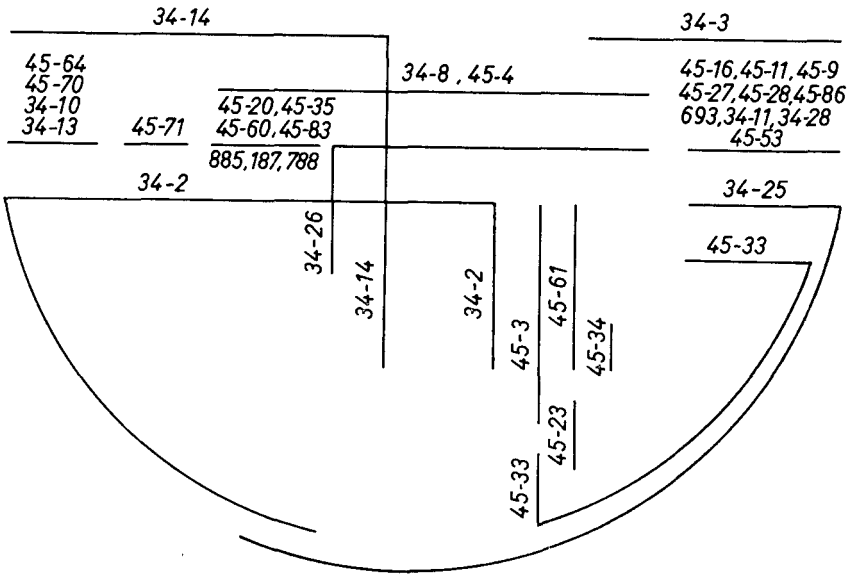

FIGURE 3.—Complementation map of the Group IV lethal alleles of *Df(2R)M-S2*[10]. (*EMS 45–72*, not shown in the complementation map, complements fully with all other Group IV lethals.)

somatic chromosome preparations nor deficiency for any proximal bands of the *2R* polytene chromosome), the locus associated with the Group IV lethals is in the vicinity of the *2R* 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 was recovered for the Group IV site. Of these lethals, 34 fell into an extensive and complex complementation map (Figure 3), while the remaining lethal, *EMS 45–72*, complemented with all others in this group. The complementation between alleles was surprisingly unambiguous (*i.e.*, heterozygous combinations of different alleles were either fully viable or completely inviable). The lethal phases for the majority of hemizygous and heterozygous combinations appeared to fall 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 3 *EMS 34–28* is shown as noncomplementary to *EMS 45–16* and *EMS 45–28* although, 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 3 as complementing with *EMS 34–26* and *EMS 45–34*, even though it does so only weakly. *EMS 45–71/EMS 34–26* heterozy-

gotes 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 exception lethal in this group, *EMS 45–72*, may represent an additional locus, but this is unclear.

Unlike the Groups A, A″ and B deficiencies, the Group A′ deficiency exhibits a dominant Minute phenotype suggesting that it is deficient for *M(2)S2* like *Df(2R)M-S2*^{10} and *Df(2R)M-S2*^{4} (Figure 1). Of all of the complementation groups of the EMS-induced lethal alleles of *Df(2R)M-S2*^{10} only Group IV is a candidate for the *M(2)S2* locus as it is the only complementation group within both the Group A′ and *M(2)S4* deficiencies. However, none of the Group IV lethals, when heterozygous with *In(2LR)SM1* exhibit the characteristic Minute phenotype, finer bristles and increased developmental time (LINDSLEY and GRELL 1968). Thus it would appear that the Group IV lethal locus does not correspond to *M(2)S2*, although it is possible that the Group IV lethals are recessive lethal alleles of the *M(2)S2* locus which are not associated with the dominant Minute phenotype.

The Group V lethals (Figure 1) are also associated with a circular complementation map (Figure 4). The Group V lethals are lethal in combination with none of the compound-second autosome detachment *2R* proximal deficiencies (Groups A, A′, A″ and B deficiencies), but are lethal in combination with *Df(2R)M-S2*^{4}. Moreover, they are viable in all combinations with the Group IV, as well as with the Groups I, II and III, lethals. These observations clearly distinguish Groups IV and V, and additionally place the locus associated with the Group V lethals distal to that associated with the Group IV. Most certainly, the Group V locus falls near the *2R* heterochromatic-euchromatic junction, but it is not clear whether it lies just within or just outside the *2R* heterochromatic block.

Complementation among the Group V lethals (Figure 4) 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 save 4, 3 involving *EMS 45–8* and 2 involving *EMS 45–26*. Although *EMS 45–8* is shown in Figure 4 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 4, 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.

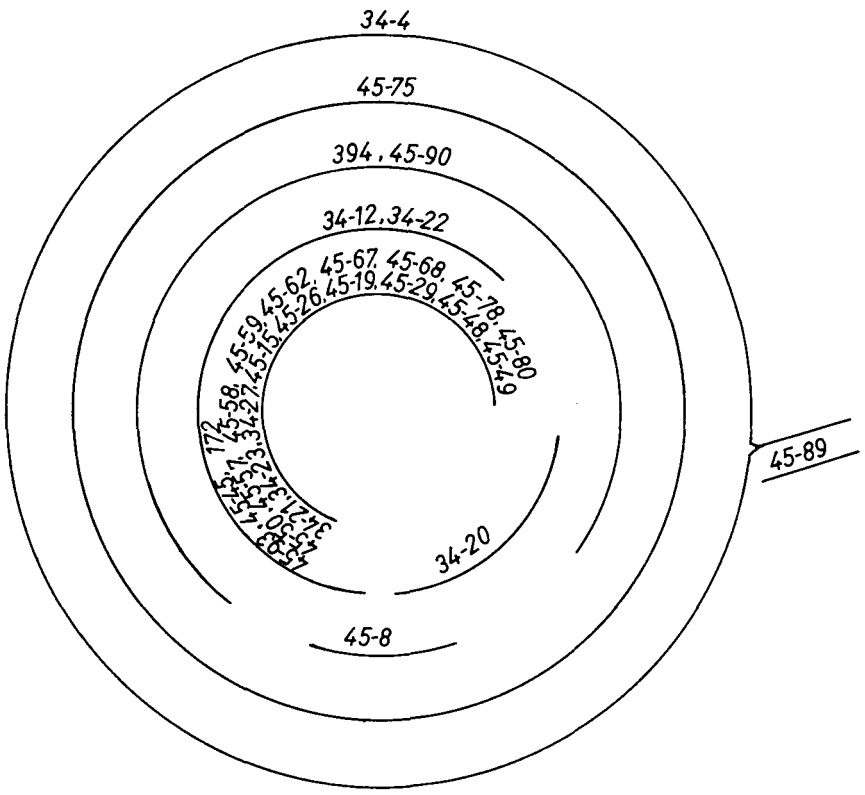

FIGURE 4.—Complementation map of the Group V lethal alleles of *Df(2R)M-S2*^{10}.

The large number of Group V lethals recovered (a total of 30) points to a relatively high EMS mutability for this locus; a characteristic it shares with the immediately adjacent Group IV locus.

The analysis of the EMS-induced lethal alleles of *Df(2R)M-S2*^{10} has revealed the existence of 3 loci within the *M(2)S10* deficiency in addition to the 3 inferred from the analysis of compound-second autosome detachments (HILLIKER and HOLM 1975). Thus there are at least 6 loci within the *M(2)10* 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 (Groups IV and V) are near the *2R* heterochromatic-euchromatic junction, and only one, Group V, falls outside the *2R* deficiencies generated through detachments of compound-second autosomes (A, A′, A″, B; Figure 1).

Of the 85 EMS-induced lethal alleles of *Df(2R)M-S2*^{10}, none are deficient for 2 or more loci; that is, all appear to be point mutations. This is consistent with the findings of LIM and SNYDER (1974). Using the same mutagenesis procedure (LEWIS and BACHER 1968), LIM and SNYDER found no deficiencies in their analysis of 82 EMS-induced lethals of the zeste-white region of the *X* chromosome of *Drosophila melanogaster* (JUDD, SHEN and KAUFMAN 1972).

It is unlikely that any of the Groups I and II lethals are deficiencies for it is clear from the compound-second autosome detachment analysis that breaks are far more likely to be proximal to the Group I site than to occur between *rl* and the Group I loci (HILLIKER and HOLM 1975). Thus a deficiency for *rl* is far more likely to be deficient for both *rl* and the Group I loci than deficient for *rl* alone. Similarly, a deficiency for a Group I locus is likely to be deficient for both Group I loci and the *rl* locus.

It is particularly unlikely that any of the Groups IV or V lethals are deficiencies. The Group IV lethals are associated with a complementation map (Figure 3) 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 Groups IV and V sites (HILLIKER and HOLM 1975); 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)S10* deficiency would appear to be point mutations implying that the loci they uncover are nonrepetitive. Moreover, the complex allele complementation maps of the Group I (*EMS 45–10*), Group IV and V loci provide further evidence that the genetic loci uncovered in the *2R* heterochromatin represent single, nonrepetitive genes. A mutant exhibiting interallelic complementation may be inferred to produce a gene product not too different from normal. It is, therefore, highly unlikely that the mutation is anything more drastic than a base pair substitution (see FINCHAM 1966). Such a mutation could be expressed only if one copy of the structural gene were present at the locus. If there were several copies of the structural gene present, the mutation would be masked.

From the preceding analysis of compound-second autosome detachments (HILLIKER and HOLM 1975) and the present analysis of EMS-induced lethal alleles of *Df(2R)M-S2*^{10} it is clear that typical (*i.e.* nonrepetitive and viability-essential) loci exist within constitutive heterochromatin, although at a very low density relative to euchromatin. The estimated gene density of the *2R* heterochromatin relative to *2R* euchromatin is 1% (*i.e.*, there are approximately one hundred times more genetic loci in a block of 2R euchromatin than in a block of *2R* heterochromatin of equivalent DNA content). 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 prometaphase somatic chromosome length to the *2R* heterochromatic block (HEITZ 1933; KAUFMANN 1934) would be represented in the polytene chromosome by a 380-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) (at the very least, the numbers of bands and genes in a chromosomal segment are in close correspondence) this leads one to an estimate of 380 genes in the *2R* heterochromatin if its gene density is equal to that of the *2R* euchromatin. Within the *2R* heterochromatin I have been able to identify 4 gene loci, thus the gene density of the *2R* heterochromatin would ap-

pear 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′:

Let us examine the results of the analysis of the genetic structure of the *2L* heterochromatic block. This analysis was accomplished through the generation of EMS-induced lethal alleles of *Df(2L)C′*. *Df(2L)C′* (Figure 5) was the largest *2L* proximal deficiency recovered by compound-second autosome detachment and genetic evidence argued that it was deficient for much of the *2L* heterochromatin (HILLIKER and HOLM 1975).

EMS mutagenesis yielded 28 lethal alleles of *Df(2L)C′* among 6467 treated and tested chromosomes. In addition there was approximately 20% 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 *lt* were recovered which survive over *Df(2L)C′* which was previously shown to be deficient for the *lt* locus.

On the basis of complementation with the *2L* proximal deficiencies described in HILLIKER and HOLM (1975), the 28 lethal alleles of *Df(2L)C′* fall into four groups (Figure 5), numbered VI, VII, VIII and IX to avoid confusion with the EMS lethal alleles of *Df(2R)M-S2*^{10}.

Let us now examine the complementation patterns of each of the four groups of lethals beginning with the most proximal, Group VI. The two Group VI lethals are lethal when heterozygous for the Groups C. C′, D and D′ *2L* proximal deficiencies but they are *lt*$^+$ (Figure 5). The Group VI lethals, *EMS 40–5* and *EMS 56–19*, were noncomplementing in combination but complemented fully when heterozygous with 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 enclosing and greatly reduced in size (to approximately ½ normal size) (HILLIKER and HOLM 1975).

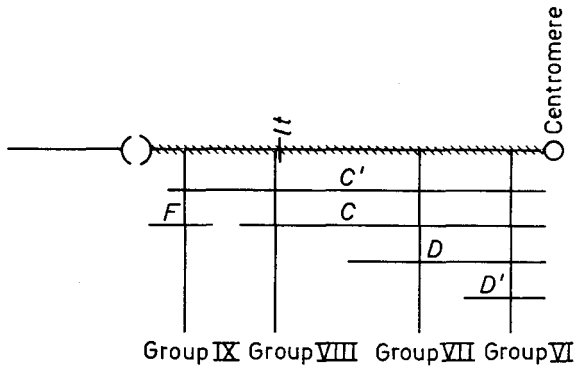

FIGURE 5.—Distribution by complementation with *2L* proximal deficiencies of EMS-induced lethal alleles of *Df(2L)C′*. Proximal heterochromatin is indicated by hatching and the secondary constriction at the *2L* heterochromatic-euchromatic junction by ( ).

The existence of the Group VII lethals confirms the previous inference that the Groups D and D′ deficiencies were genetically distinct 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 (Figure 5). Clearly, the Group D deficiencies have a greater distal extent than the Group D′ deficiencies, as was previously inferred (HILLIKER and HOLM 1975). Inspection of the Group VII lethal complementation pattern leads one to conclude that possibly two loci are associated with the four Group VII lethals. *EMS 56–24* complemented fully with the other three 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 this latter group were completely inviable when hemizygous and in all *inter se* combinations of the three lethals. A semilethal allele of this group also was recovered, *EMS 40–22*. *EMS 40–22* hemizygotes were of approximately 20% viability with these surviving hemizygotes 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 eclosing later than their sibs. *EMS 40–22* complemented fully with all other *2L* lethals including *EMS 56–24*, the remaining Group VII lethal. Thus, the Group VII lethals comprise two complementation groups with no overlap.

The Group VIII lethals are uncovered only by the Groups C and C′ deficiencies and therefore define a region distal to the Groups D and D′ deficiencies and proximal to the Group F deficiencies (Figure 5). Group VIII is divided into three distinct, nonoverlapping complementation subgroups (Figure 6), one of which corresponds to the *lt* locus. In addition to 5 visible alleles of *lt*, which were hemizygous viable and of *lt* phenotype, 3 lethal alleles of the *lt* locus were recovered, *EMS 40–12*, *EMS 40–17* and *EMS 56–3*. The lethal alleles of *lt* were of light

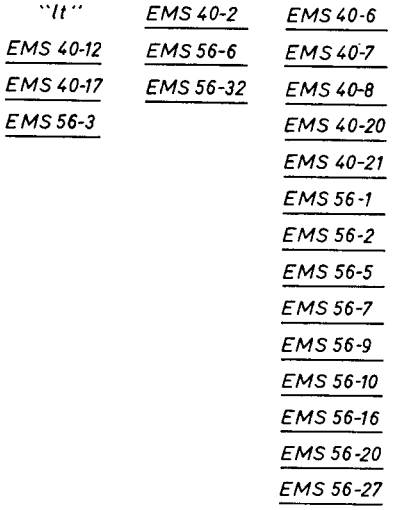

FIGURE 6.—Complementation map of the Group VIII lethal alleles of *Df(2L)C′*.

phenotype when heterozygous with a nonlethal *lt* allele, as well as lethal in combination with one another. Hemizygotes for the *lt* lethal alleles die in the late pupal stage of development. Thus, it would appear that *lt* is a viability-essential locus. The two other Group VIII complementation subgroups are the *EMS 40–2* complex of three lethals and the *EMS 40–6* complex of fourteen lethals. (Figure 6). The *EMS 40–2* complex consists of *EMS 40–2*, *EMS 56–6* and *EMS 56–32*. All three lethals of the *EMS 40–2* complex are lt^+ and viable in combination with the 25 other *Df(2L)C′* lethal alleles. The significance of the fact that 14 of the 28 EMS-induced lethal alleles of *Df(2L)C′* fall at the *EMS 40–6* site is unclear. Moreover, 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, whose relative order remains unknown.

The Group IX lethals are the most distal of the *2L* lethals. The group consists of two noncomplementing lethals, defined on the basis of lethality in heterozygotes with the C′ and F deficiencies. Moreover, they are distinguished from Group VIII by their viability with the C deficiency and their lt^+ phenotype. Hemizygotes with the C′ and F deficiencies for *EMS 40–18* and *EMS 56–9* are associated with a rather interesting lethal phenotype. These genotypes 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 pupal 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 distinct complementation groups within the deficiency. 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 the loci in the *2L* heterochromatin. *Df(2L)C′* is not deficient for any proximal bands in the euchromatic *2L* polytene chromosome (at least any distal to 40C) nor is it deficient for the secondary constriction at the *2L* heterochromatic-euchromatic junction. In addition, the *C(2R)VKl,Dp(2L)lt⁺, bw* chromosome, which, as the recovery of the Group F deficiencies demonstrates (see text and Figure 4 of HILLIKER and HOLM 1975), 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 nor any *2L* polytene proximal bands. Further, although the Group F deficiencies on the basis of their Minute Phenotype, possibly *M(2)H* (LINDSLEY and GRELL 1968), would appear to have a greater distal extent than the non-Minute *Df(2L)C′*, neither of the two Group F deficiencies is deficient for proximal bands in the *2L* polytene chromosome distal to 40C and neither of them lacks the *2L* secondary constriction.

The four loci associated with the Groups VIII and IX lethals may lie in the immediate vicinity of the *2L* heterochromatic-euchromatic junction as only one

deficiency deficient for the Group IX lethal locus, the Group C′ deficiency, and two 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 (HILLIKER and HOLM 1975); however, the observation that *C(2R)VKl,Dp-(2L)lt+,bw* is not duplicated for any portion of the *2L* secondary constriction leads one to conclude that the Group VIII loci are definitely within the *2L* heterochromatin. Eight detachments, the Groups C, C′ and 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 16 *C(2L)SH3+; C(2R)SH3+* detachments were deficient, the Groups C, C′, D and D′ deficiencies) are well within the *2L* proximal heterochromatin.

Consistent with the findings for EMS-induced lethal alleles of *Df(2R)M-S2[10]*, none of the lethal alleles of *Df(2L)C′* induced by EMS appear to be deficiencies, none affecting more than one locus.

DISCUSSION

This study completes our genetic dissection of the proximal region of chromosome *2* of *Drosophila melanogaster*. Our previous report described the analysis of the chromosome *2* proximal region through the detachment of compound autosomes (HILLIKER and HOLM 1975). The present report describes the further analysis of this region through the induction with ethyl methanesulphonate (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 is observed for the bobbed locus in the *X* chromosome heterochromatin, which is associated with the prominent nucleolar secondary constriction (RITOSSA, ATWOOD and SPIEGELMAN 1966). Further, the second chromosome chromocentral loci appear to be nonrepetitive genes, *i.e.* only one copy of the structural gene is present at each locus. Additionally, lethal alleles of the chromosome *2* heterochromatic loci uncovered in this study have late-larval and pupal lethal phases. Finally, although genes have been demonstrated within the 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 that have heretofore been interpreted as conclusive evidence of the complete genetic inactivity of constitutive heterochromatin (reviewed in 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 repeated nucleotide 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 segment cannot be demonstarted biochemically, only genetically, as has been accomplished here for the Drosophila second chromosome heterochromatin.

The analyses of the EMS-induced lethal alleles of *2L* and *2R* proximal deficiencies have confirmed that the EMS mutagenesis procedure of LEWIS and BACHER (1968) does not induce chromosomal deficiencies. LIM and SNYDER (1974) arrived at this 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 inasmuch as it does not induce deletions in Drosophila heterochromatin or euchromatin.

In addition to confirming that EMS does not generate chromosomal deletions, 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-S*^{10}. For example, if any one lethal was missing from the *EMS 45–10* complex of the Group I lethals (Figure 2), then the complementation map would resemble that of three overlapping deficiencies. This would imply that at least two genetic loci were associated with the *EMS 45–10* complex. Another example is that of the Group V lethals (Figure 4). 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 suggest that 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 conclusion, while it is clear from this study that heterochromatin is not genetically inert, it is evident that gene density in heterochromatin is very low relative to euchromatin. Perhaps the genetically inactive regions serve to provide a necessary physical environment for the normal expression of genes intercalated in and adjacent to heterochromatic regions, possibly by the association of the chromocentre with the nuclear membrane. However, although heterochromatin is not completely devoid of genes, the principal functions of the bulk of heterochromatin, if any, remain undefined.

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