Genetic Analysis of the Heterochromatin of Chromosome *3* in *Drosophila melanogaster*. I. Products of Compound-Autosome Detachment

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ABSTRACT

The heterochromatin of the third chromosome is the largest uncharacterized region of the *Drosophila melanogaster* genome, and the last major block of *D. melanogaster* heterochromatin to be thoroughly analyzed. In the present study, this region was genetically dissected by generating and analyzing a series of attached, detached and reattached third chromosomes. Separate detachment experiments were conducted for all 12 possible combinations of four newly synthesized sister-strand compound-*3L* and three newly synthesized sister-strand compound-*3R* chromosomes. A total of 443 recessive lethal detachment products carrying putative heterochromatic deficiencies were tested for complementation in a several-stage complementation analysis. The results revealed the presence of seven separable vital regions in the heterochromatin of chromosome *3*. Attempts to reattach deficiency-carrying detachment products established that six of these vital regions are on the left arm, but only one is on the right arm. An analysis of the types and frequencies of detachment-product deficiencies generated in each detachment experiment permitted the genetic characterization of the progenitor compounds. It was also possible to determine the proximal-distal orientation of the genes on each arm, and to identify possible breakpoints for each lethal detachment product produced. The results of this study suggest that vital genes in the heterochromatin of the third chromosome are not randomly distributed between, nor within, the heterochromatic blocks of the left and right arms.

CHROMOSOME *3* of *Drosophila melanogaster* contains the last major uncharted block of heterochromatin to be genetically analyzed in this species. Heterochromatin is of two types, facultative and constitutive (BROWN 1966), but only constitutive is present in the chromosomes of Drosophila. Unlike the euchromatic regions of the genome, heterochromatin remains condensed and darkly stained throughout the cell cycle of most cells. It occupies identical positions on homologous chromosomes and in Drosophila accounts for the proximal half of the *X* chromosome, the entire *Y*, the proximal 1/5 to 1/4 of the arms on the two major autosomes, and most of the small fourth chromosome. In total, heterochromatin represents approximately 28% of the total Drosophila genome (PEACOCK *et al.* 1973).

There is a striking correlation between regions of the genome with little or no genetic activity and regions of heterochromatin. The DNA composition of constitutive heterochromatin would also argue that little genetic activity could occur in these regions. Constitutive heterochromatin is greatly enriched in satellite DNA, which is composed of very short nucleotide sequences present in hundreds of thousands of copies per genome (YASMINEH and YUNIS 1969; RAE 1970; JONES and ROBERTSON 1970; PARDUE and GALL 1970; PEACOCK *et al.* 1973; GALL and ATHERTON 1974). PEACOCK *et al.* (1973, 1977a) have shown that the constitutive heterochromatin of *D. melanogaster* and other organisms is almost entirely composed of long, homogeneous blocks of individual satellite DNA sequences tandemly arranged in a chromosome-specific order. The estimated amount of satellite DNA in each block of heterochromatin almost completely accounts for the total amount of DNA present (PEACOCK *et al.* 1977b), suggesting that few, if any, other types of DNA sequences reside within *D. melanogaster* heterochromatin. This view was supported by the discovery of junction DNA molecules that covalently link adjacent blocks of satellite DNA (BRUTLAG *et al.* 1977). While a few nonsatellite sequences appear to be in heterochromatin, only trace amounts of satellite DNA are found outside the heterochromatic regions (PEACOCK *et al.* 1977a; COHEN and BOWMAN 1979).

Even though the molecular and structural properties of heterochromatin indicate a paucity if not a complete absence of gene expression, a number of genetic functions have been mapped to the heterochromatin of *D. melanogaster* (see review by HILLIKER, APPELS and SCHALET 1980). The nucleolus organizer regions in both the *X* and *Y* heterochromatin contain the tandemly repeated genes for rRNA (RITOSSA and SPIEGELMAN 1965). Although these genes are located at the large secondary constrictions in the heterochromatin, they retain many of the properties of hetero-

chromatin when separated from the heterochromatic region (HILLIKER and APPELS 1982). Male fertility in *D. melanogaster* requires the presence of six fertility factors on the heterochromatic *Y* chromosome (BROUSSEAU 1960; KENNISON 1981; HAZELRIGG, FORNITI and KAUFMAN 1982; GATTI and PIMPINELLI 1983). Each fertility factor is involved in a specific step in sperm development (HARDY, TOKOYASU and LINDSLEY 1981) and seems to code for a different protein required for sperm development and fertility (GOLDSTEIN, HARDY and LINDSLEY 1982). The recovery of EMS-induced (WILLIAMSON 1970, 1972) and temperature-sensitive (AYLES *et al.* 1973) mutations of male fertility factors suggest that they are single-copy, transcribed genes. However, on the basis of an extensive break-point analysis, GATTI and PIMPINELLI (1983) have suggested that at least some of the *Y* fertility factors may have an enormous physical size, each containing up to several thousand kilobases of DNA. In addition to the six fertility factors there is a specific segment on the long arm of the *Y* required for normal sperm development. Deletion of this segment results in crystal formation in primary spermatocytes, but not sterility (HARDY *et al.* 1984; LIVAK 1984).

Other genetic properties have been mapped to specific regions of *D. melanogaster* heterochromatin, although it is not clear whether transcribed genes are involved. The chromosomal regions responsible for nucleolar dominance (DURICA and KRIDER 1978) and compensation response (PROCUNIER and TARTOF 1978) are located in the *X* chromosome heterochromatin. A segment of the *X* heterochromatin also interacts with a family of recessive maternal mutations including *abnormal oocyte* (SANDLER 1979, 1972, 1977; PARRY and SANDLER 1974; PIMPINELLI *et al.* 1985). Extra copies of a specific segment of *X* heterochromatin as well as two segments of the *Y*, can partially compensate for the maternal mutant defect in *abnormal oocyte* progeny (SANDLER 1970; PIMPINELLI *et al.* 1985). Finally, elements involved in the segregation distortion (SD) phenomenon have been mapped to the heterochromatin of chromosome *2* (GANETSKY 1977; BRITTNACHER and GANETSKY 1984; SHARP, HILLIKER and HOLM 1985), and recently MCKEE and LINDSLEY (1987) report that the heterochromatic sites on the *X* responsible for sex chromosome pairing are very closely linked and probably identical with the sites required for normal sperm development and for fertility in the presence of duplicated *Y*s.

Proximal deficiencies generated through the detachment of compound-*2* chromosomes in females revealed the presence of at least six genetic loci within the centromeric heterochromatin of chromosome *2* (HILLIKER and HOLM 1975). Further analysis of this region by the induction with ethyl methane-sulfonate of recessive lethal and visible mutations allelic to the deficiencies of the heterochromatic region of chromosome *2* revealed the presence of 13 nonrepetitive genes, seven to the left and six to the right of the centromere (HILLIKER 1976). Cytological examinations of the detachment-product deficiencies in chromosome *2* indeed demonstrated that the deficiencies were restricted to the heterochromatic regions.

However, the euchromatin-like genes shown to be in chromosome *2* heterochromatin do not seem to exist in the heterochromatin of the *X* or *Y* chromosome (LINDSLEY, EDINGTON and VON HALLE 1960; SCHALET and LEFEVRE 1973; HILLIKER and APPELS 1982; KENNISON 1981; GATTI and PIMPINELLI 1983) (see also review by HILLIKER, APPELS and SCHALET 1980). These findings raise the possibility that heterochromatin in *D. melanogaster* may be heterogeneous with respect to genetic activity. Since the heterochromatin of the sex chromosomes has a very different genetic composition than the heterochromatin of at least one of the major autosomes, it would be of significant interest to determine if the genetic content of chromosome *3*, the other major autosome, conforms to this dichotomous pattern. The results of BALDWIN and SUZUKI (1971) which introduced the technique of generating proximal deficiencies through the detachment of compound autosomes, suggested that chromosome *3* heterochromatin does contain essential genes. However, there was insufficient information about the number, distribution, repetitiveness and nature of genes in chromosome *3* heterochromatin to permit a valid comparison between the heterochromatic gene content of the two major autosomes.

In the present study we analyzed over 440 reconstituted third chromosomes generated through the radiation-induced detachment of 12 different combinations of *C(3L)* and *C(3R)*. These compound autosomes were newly generated as sister-chromatid attachments from a recently established isogenic-*3* strain to avoid the accumulation of recessive lethals, especially near the centromere where the frequency of homozygosity is very low (PARKER 1954). Some of these compounds carried duplications for the proximal heterochromatin on the opposite arm and some were heterozygous for deficiencies. Although these properties were not known prior to recovering and analyzing the products of detachment, they provided the essential genetic complexity for producing polar, nonpolar and centromere-spanning deficiencies that uncovered vital genetic loci in the heterochromatin of chromosome *3*. Unlike the studies on chromosome *2* (HILLIKER and HOLM 1975) where visible genetic markers were uncovered by deficiencies to the left or to the right of the centromere, no genetic markers had been previously identified within the heterochro-

matic regions of chromosome 3. Consequently, the construction of sister-chromatid compound-3 chromosomes was attempted with representative detachment products carrying deficiencies. This approach made it possible to assign each of the newly discovered genetic loci either to the right or to the left of the centromere.

The procedures described above have made possible an extensive genetic investigation of the proximal heterochromatin of chromosome 3 confirming and extending the original findings of BALDWIN and SUZUKI (1971). The results of this study reveal that at least six vital genetic loci occupy sites in the heterochromatin to the left of the centromere and that at least one locus is in the heterochromatin to the right.

MATERIALS AND METHODS

Genetic markers, rearranged chromosomes and experimental conditions: Four standard and two compound-3 strains of *D. melanogaster* were used in this study. All newly generated compound third chromosomes and their detachment products carried the proximal recessive markers *radius incompletus* (*ri*) on the left arm and *pink-peach* (p^p)) on the right. Detachment products were balanced over *In(3LR)TM3, ri p^p sep Sb bx^{34e} e^s Ser* (hereafter referred to as *TM3*), which has been maintained in a stock heterozygous for the dominant mutation *Ly*. The proximal position of putative deficiencies was confirmed by crossover analysis using the two genetically marked third chromosomes *st in ri eg* and *st eg Ki* or a derivative. Two compound-3 strains, *C(3L)VT1,se*; *C(3R)VK1,e^s* and *C(3L)VH3,st*; *C(3R)SH19*, +, were used to rescue the newly generated compound autosomes. Further information on these mutations and rearranged chromosomes can be obtained from LINDSLEY and GRELL (1968). Unless otherwise stated, all experimental crosses were carried out at 24 ± 1° on standard Drosophila medium prepared from cornmeal, yeast, sucrose, dextrose and agar with propionic acid added as a mold inhibitor.

Synthesis of new compound-3 chromosomes: All compound autosomes used for detachments were recovered as sister-chromatid attachments generated from a strain isogenic for the *ri p^p* third chromosome. This isogenic *ri p^p* strain was obtained by mating a single *ri p^p/TM3* male to *TM3/Ly* females, and then mating siblings of the *ri p^p/TM3* progeny to isolate a homozygous *ri p^p* stock. *ri p^p* males were collected and mated to virgin *TM3/Ly* females. Approximately 1500 virgin F_1 *ri p^p/TM3* females were collected and treated with 2000 rad of γ-radiation from a ^{60}Co source. The irradiated females were mated in groups of 25 to *C(3L)VT1,se*; *C(3R)VK1,e^s* males in half-pint milk bottles. The parents were cleared after 10 days. New compounds generated in females heterozygous for *TM3* could be formed only through the attachment of sister chromatids of the noninverted *ri p^p* homologue. The few surviving progeny either carried a newly formed compound third, or arose as a product of a nondisjunctional event. All *ri e^s* offspring carried a newly formed sister-strand *C(3L)* chromosome, while *se p^p* individuals carried a new sister-strand *C(3R)* chromosome. Separate stocks were established for each possible combination of newly synthesized compound thirds. All compound thirds generated for this study have been labeled with the letters *VM* followed by a number specifying the compound in the series.

Recovery of detachment products: A separate detachment experiment was performed for each of twelve lines carrying a different combination of new *C(3L)* and *C(3R)* chromosomes. In each experiment, 2000 virgin compound-3 females were treated with 2200 rad of gamma radiation and mated in groups of fifty to *TM3/Ly* males in half-pint bottles. The parents were cleared after 10 days, and offspring were collected at 18° for the next 20 days. Virgin females and males carrying detachment products recovered over *TM3*, and males carrying detachment products recovered over *Ly* chromosome, were established in stock over *TM3*.

Lethality tests: Each detachment product was tested for homozygous lethality by mating males and females carrying the same detached *ri p^p* chromosome balanced over *TM3*. All lethality tests were carried out in duplicate in shell vials with two pairs of flies per vial. Recessive lethals were identified as the total absence of *ri p^p* progeny. If the detachment product was homozygous viable, then *ri p^p/TM3* heterozygotes and homozygous *ri p^p* flies were expected in approximately a 2:1 ratio. All offspring in each vial were counted to ensure that semilethals would be detected. Detachment products carrying recessive lethals were maintained in stock over *TM3*. Approximately 150 nonlethal detachment products were also saved.

Complementation tests: To make possible the analysis of a large number of detachment products without requiring an inordinate number of crosses, complementation testing was done in several stages. First, lethal detachment products from five of the 12 detachment experiments were selected for complementation analysis. For each of these five sets all *inter se* crosses were tested for lethality. From these tests, a separate complementation map was produced for each of the five sets of detachment chromosomes.

The second stage of the complementation analysis was to test between complementation groups of the five detachment experiments. Several detachment products were selected from every complementation group in each of the five experiments. These chromosomes were then pooled and tested in all *inter se* combinations, producing an accumulated complementation map for the five original experiments. Further complementation tests were carried out within complementation groups on the accumulated map wherever necessary to resolve any ambiguities.

In the third stage of the complementation analysis tests were carried out with all lethal detachment products from the seven remaining experiments against a tester chromosome from each complementation group on the accumulated map. From the results of these crosses, all the detachment products of the seven remaining experiments were assigned to a complementation group on the overall map. Finally, some random complementation tests were conducted within each complementation group to ensure that none of the complementation groups could be further subdivided.

In each stage of the complementation analysis described above, all tests involved crossing a pair of males carrying one lethal balanced over *TM3* to a pair of females carrying a second lethal over *TM3*. All crosses were carried out in shell vials. Reciprocal crosses were performed for most complementation tests. Crosses between two noncomplementing lethals produced only *ri p^p/TM3* progeny. In crosses between complementing lethals, *ri p^p/TM3* and *ri p^p/ri p^p* progeny were recovered in an approximate ratio of 2:1.

Recombination mapping: The proximal position of putative deletions carried by the detachment products was confirmed by crossover analysis. A single detachment chromosome from each complementation class was tested in two recombination mapping experiments. The first crossover

analysis determined if the deficiency on the tester chromosomes was to the left or to the right of *eagle*, the most proximal visible marker on the left arm of chromosome *3*. Females heterozygous for a *ri p*^{p} detachment product over the marker chromosome *st eg Ki* were mated to homozygous *st in ri eg* males. F_1 offspring were screened for crossovers between *ri* at map position 47.0 and *eg* at map position 47.3. Recombinant progeny with a scarlet phenotype carried the heterochromatic region of the detachment chromosome, the reciprocal *ri eg Ki* product did not. Both classes of recombinant products were tested for lethality over the original detachment product. If the recombinant chromosome recovered in the scarlet progeny failed to survive over the detachment product, then the deficiency carried by the detachment product was to the right of the *ri-eg* region. Conversely, if the reciprocal *ri eg Ki* product failed to survive, the deficiency was in the left arm euchromatin.

A comparable experiment was conducted for the right arm. Females carrying the detachment product over the marker chromosome *eg Ki* were mated to homozygous *ri p*^{p} males. Crossovers in the region between *Kinked* at map position 47.6 and *pink-peach* at map position 48.0 were recovered. Progeny with a Kinked, pink-peach phenotype carried a recombinant chromosome containing most of the right arm euchromatin, whereas progeny with a radius incompletus phenotype carried the reciprocal recombinant product containing the entire left arm and the right-arm heterochromatin of the detachment-product chromosome. If the recombinant chromosome which included the entire left arm and the heterochromatin of the right arm were lethal over the original detachment product, the detachment product deficiency was to the left of the *Ki p*^{p} region. If the reciprocal recombinant product failed to survive over the complete detachment, the deficiency was distal to the *Ki p*^{p} region on the right arm.

A putative detachment product deficiency was assigned to the proximal region of chromosome *3* with certainty if it mapped to the right of the *ri eg* region and to the left of the *Ki p*^{p} region.

Reattachments: To determine whether the proximal deficiencies recovered as lethal-bearing products of compound-*3* detachment were to the left or to the right of the centromere, an attempt was made to regenerate compound autosomes by reattaching sister chromatids of the detachment products from several complementation classes. Virgin females heterozgyous for the deficiency-bearing detachment chromosome over *TM3* were irradiated and mated to *C(3L)VH3,st; C(3R)SH19,+* males. For each detachment product tested, 2500 females were treated with 2500 rad and an additional 1250 females were treated with 4000 rad of γ-radiation. Progeny with a radius incompletus phenotype carried a newly formed sister-strand *C(3L)*; and those with a scarlet, pink-peach phenotype carried a new sister-strand *C(3R)*. Only *C(3L)*'s were expected from detachment products with a right-arm deficiency, and vice versa.

Models representing the generation of polar, nonpolar and centromere-spanning heterochromatic deficiencies: From previous studies (Holm 1976; Hilliker, Holm and Appels 1982) we recognize that a compound autosome can carry a duplication for proximal loci from the opposite arm, a deficiency for proximal, vital loci of the same arm, both or neither. In Figure 1 we have presented four possible configurations of hypothetical heterochromatic loci in compound-*3L* chromosomes arising as a consequence of anisobrachial interchanges between sister chromatids. Assuming that genetic loci do reside within the heterochromatic regions of chromosomes, to obtain a full understanding of the distribution and relative position of these genes, it is essential

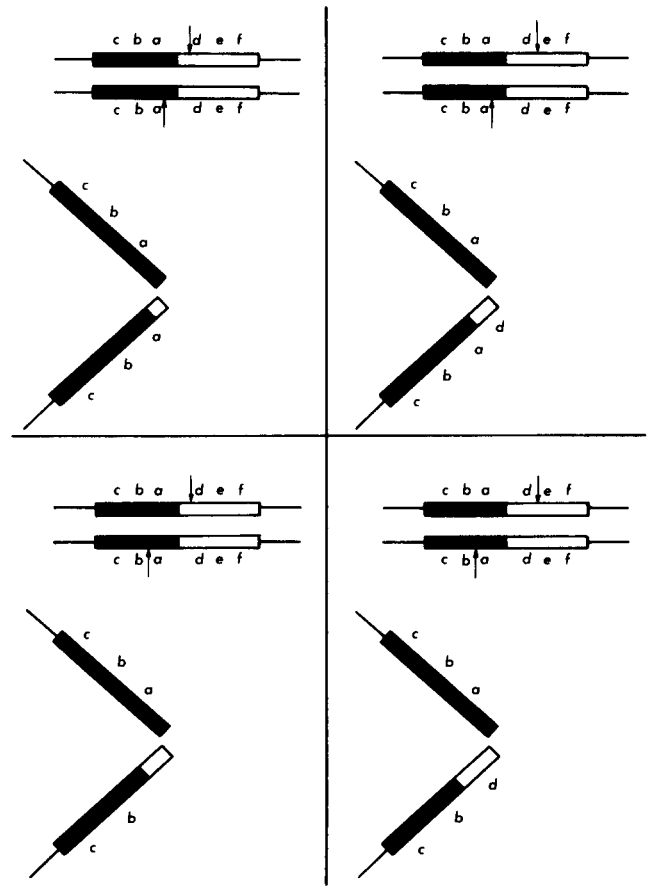

Figure 1.—Models showing the synthesis of the four possible types of compound autosomes. *C(3L)* chromosomes formed by sister-strand attachments are used as examples. Radiation-induced breaks are indicated by arrows, *3L* heterochromatin as solid blocks, and *3R* heterochromatin as open blocks. The symbols *a*, *b*, and *c* represent vital loci in wild-type left heterochromatin; while *d*, *e*, and *f* designate loci in wild-type right heterochromatin. (Top left) Synthesis of a *C(3L)* chromosome carrying no proximal deficiencies or duplications of vital loci. (Top right) Synthesis of a *C(3L)* chromosome carrying a proximal duplication (*d*) of *3R* heterochromatin. (Bottom left) Synthesis of a *C(3L)* chromosome carrying a proximal deficiency of *3L* heterochromatic region containing locus *a*. (Bottom right) Synthesis of a *C(3L)* chromosome carrying both a proximal *3R* duplication and *3L* deficiency.

to know the nature of the progenitor compound autosomes. However, unlike chromosome *2* where two, recessive, visible genetic markers were available to help identify some of the properties of the compound autosomes, no identifying markers for the heterochromatic regions were available for chromosome *3*. Therefore, it was not immediately possible to assign any of the newly generated compound-*3* chromosomes to any of the four classes represented in Figure 1. Any deficiency of vital loci generated on a compound autosome will be masked by the normal alleles on the homologous arm. It is assumed also that offspring with compounds carrying a duplication are phenotypically normal. Consequently, a compound autosome can only be characterized by working backward from the pattern of detachment products generated. Since the frequency and type of lethal detachments produced from a given pair of compound autosomes is a function of the interaction of the classes of both compounds within the pair, each compound autosome must be detached in several different combinations before it can

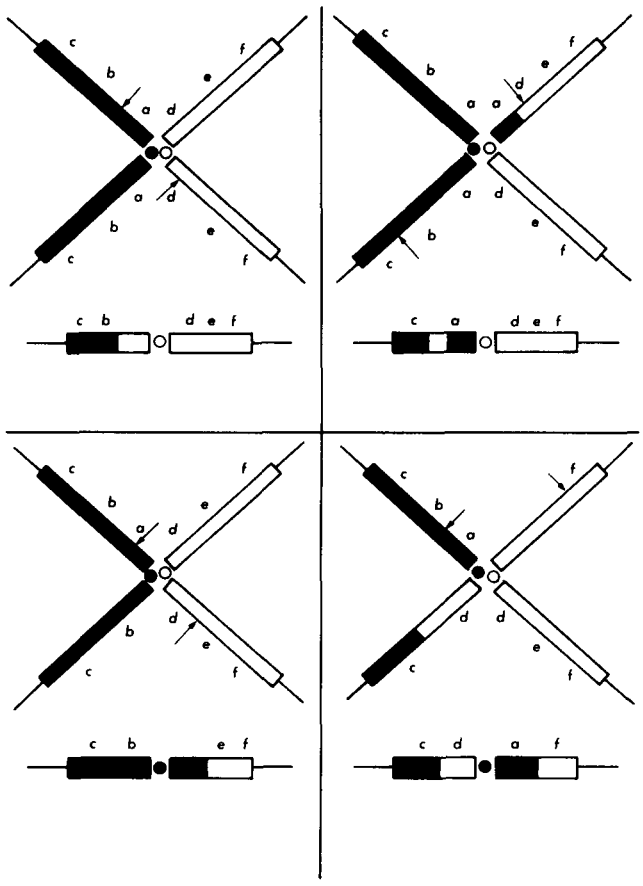

FIGURE 2.—Models showing the generation of four possible types of proximal deficiencies carried by detachment products. Irradiation-induced breaks are shown as arrows, *3L* heterochromatin as solid blocks, and *3R* heterochromatin as open blocks. The symbols *a*, *b*, and *c* represent loci found in wild-type left heterochromatin; while *d*, *e*, and *f* designate loci in wild-type right heterochromatin. (Top left) Production of a polar deficiency (*a*) of the left arm from progenitor compounds carrying no duplications or deficiencies of vital loci. (Top right) Production of a nonpolar deficiency (*b*) of the left arm from progenitor compounds carrying a duplication of locus *a* in the left-arm heterochromatin. (Bottom left) Production of a centromere-spanning deficiency (*a* and *d*) from progenitor compounds carrying a deficiency of left arm heterochromatin. (Bottom right) Production of a more complex deficiency (*b* and *e*) from progenitor compounds carrying duplications and deficiencies. Note the relative positions of the most proximal loci *a* and *d*.

be properly characterized. In the present study, these objectives were met by undertaking separate detachment experiments for all 12 possible combinations of three different *C(3R)* chromosomes and four different *C(3L)* chromosomes.

From each type of compound autosome, as represented in Figure 1, a different pattern of detachment products (reconstituted standard autosomes) can be obtained. Many classes of detachment products can be produced, as shown in Figure 2, but only those with deficiencies of vital genetic loci can be readily identified. The types and frequencies of deletions generated depend on the class of progenitor compounds. From compound autosomes carrying no duplications or deficiencies of vital loci, only polar deficiencies, starting from the centromere, will be recovered (top left model in Figure 2). Nonpolar deficiencies will be obtained only from detaching compound autosomes in which at least one carries a duplication or a deficiency. Centromere-spanning deficiencies will result from progenitor compound autosomes in which at least one is hemizygous for a vital locus in the proximal heterochromatin.

The frequency of lethal detachment products generated is also partially determined by the class of the progenitor compounds. For each detachment event, there is a reciprocal detachment which would be expected to occur with an equal frequency. Each time a compound autosome carrying a deficiency for a given region is detached, one of the two reciprocal detachment products will carry the deficiency. Therefore, a minimum of 50% of the detachment products from a deficiency-carrying compound will be deficient for the same region. If no deficiency is carried by the progenitor compounds, the proportion of detachment products deficient for a specific region cannot exceed 50%. The frequency of detachment products carrying any deficiency will also be determined by whether or not the progenitor compounds carried duplications as well.

RESULTS

Synthesis of compound-*3* chromosomes: Six sister-chromatid compound-*3L* and three sister-chromatid compound-*3R* chromosomes were recovered from approximately 1500 irradiated females. Each compound third was assigned an α-numeric code as recommended by HOLM (1976). The three *C(3R)* chromosomes were designated *C(3R)VM1,p^p* to *C(3R)VM3,p^p*; and the six *C(3L)*chromosomes were designated *C(3L)VM1,ri* to *C(3L)VM6,ri*. All three *C(3R)* chromosomes and the first four *C(3L)* chromosomes were used in the detachment experiments. Twelve separate lines were established from all possible combinations of these compound chromosomes.

Recovery of detachment products: A separate detachment experiment was undertaken for each of the 12 compound-*3* combinations. In each experiment, 2000 virgin females from a compound-*3* strain were irradiated and mated to *TM3/Ly* males. The offspring recovered were heterozygous for the detachment product over the *TM3* or the *Ly* chromosome. The number of detachment products rescued in each experiment is shown in Table 1. Owing to the relatively low recovery of products in the initial cross with *C(3L)VM3; C(3R)VM2*, experiment 6 was repeated.

It will be noted that from the four detachment experiments involving *C(3R)VM2* substantially fewer reconstituted thirds were recovered than from any of the other crosses. To test whether this effect was a result of reduced fertility in stocks carrying *C(3R)VM2*, fecundity tests were carried out for each of the 12 compound lines. Two-day-old virgin females from each line were treated with 2200 rad and mated to males carrying a pair of compound thirds. Six bottles of 25 females and 10 males were examined for each compound line. The fecundity of stocks carrying the *C(3R)VM2* chromosome proved not to differ significantly from that of the other compound stocks. An alternative explanation was that the *C(3R)VM2*

TABLE 1

Number of reconstituted third chromosomes recovered from each compound-*3* detachment experiment[a]

Expt. No.	*C(3L)*	*C(3R)*	No. of detachments recovered
1	*VM2*	*VM1*	218
2	*VM4*	*VM2*	108
3	*VM3*	*VM1*	166
4	*VM1*	*VM1*	201
5	*VM1*	*VM2*	112
6A	*VM3*	*VM2*	80
6B	*VM3*	*VM2*	98
7	*VM4*	*VM3*	178
8	*VM4*	*VM1*	144
9	*VM2*	*VM2*	93
10	*VM3*	*VM3*	218
11	*VM1*	*VM3*	163
12	*VM2*	*VM3*	238

[a] In each experiment, 2000 compound-*3* females were treated with 2200 rad of γ-radiation and mated with *TM3/Ly* males.

TABLE 2

Results of lethality tests with reconstituted third chromosomes from each detachment experiment

Expt. No.	*C(3L)*	*C(3R)*	No. of lethals	No. of nonlethals	Percent lethals[a]
1	*VM2*	*VM1*	40	79	33
2	*VM4*	*VM2*	53	31	63
3	*VM3*	*VM1*	66	38	63
4	*VM1*	*VM1*	38	116	25
5	*VM1*	*VM2*	42	47	47
6	*VM3*	*VM2*	42	16	72
7	*VM4*	*VM3*	62	31	67
8	*VM4*	*VM1*	33	109	23
9	*VM2*	*VM2*	39	32	55
10	*VM3*	*VM3*	62	29	68
11	*VM1*	*VM3*	50	51	50
12	*VM2*	*VM3*	41	39	51

[a] The percent of all detachment products tested that carried recessive lethals.

chromosome carried a massive deficiency of proximal heterochromatin, and thereby offered a smaller target to γ-rays. Subsequent findings reported below failed to substantiate this proposal. Nevertheless, the possibility that some intrinsic property of compound autosomes may influence their detachment frequencies deserves further attention.

Detachment products recovered in males or females over *TM3* and in males over *Ly* were maintained in stock balanced over *TM3*. Each detachment product was assigned a code number which identified the experiment, and hence the compounds from which they were derived, and the detachment product. For example, *2–10* is the tenth detachment product recovered from experiment 2.

Lethality tests: The results of lethality tests with the detachment products tested from each experiment appear in Table 2. No apparent semilethality was detected and no sterility was observed. It can be seen from the data that the percentage of lethal-carrying detachment products varied considerably from experiment to experiment. In three experiments, less than 35% of the detachment products carried lethals; while in five others, greater than 60% of the chromosomes were homozygous lethal. The other four experiments were within 5% of 50% lethals. These differences in lethal frequencies likely reflect different classes of progenitor compounds. At least 25 lethal detachment products from each experiment were maintained in stock and used in the complementation analysis. A total of approximately 150 nonlethal detachment products were also saved.

Complementation analysis: A total of 443 lethal detachment products from the 12 separate experiments were tested for complementation following the several-step procedure described in MATERIALS AND METHODS. First, a separate complementation map was produced for experiments 1, 2, 3, 4, and 10 by testing the lethal detachment products of each experiment in all *inter se* combinations. Testing between complementation groups from the different experiments produced an accumulated complementation map for the five experiments. Finally, an accumulated complementation map for all twelve experiments was produced by testing all the lethal detachment products from the remaining seven experiments against tester chromosomes from each complementation group in the five-experiment complementation map.

Figure 3 represents the accumulated complementation map of the lethal detachment products from all 12 experiments. Each complementation group is represented by a solid bar; complementation groups shown to overlap fail to complement. Above each bar the complementation group is designated by the code number of a representative detachment product. The number under the bar represents the number of chromosomes that fall into that particular complementation group.

As shown in Figure 3, 18 complementation groups were identified with as many as 146 and as few as 1 detachment product per group. This initial complementation map suggested that at least eight, distinct, vital loci were uncovered by the putative deficiencies on the detachment chromosomes. Small complementing deficiencies specific for seven of these regions were indicated, and the presence of an eighth region between the *3–30* and *3–9* groups was implied by the complementation pattern. The two complementation groups shown as two solid blocks joined by a dotted line were interpreted as detachment products that carried double deficiencies, one on each side of the centromere. Such centromere-spanning double deficiencies could only be generated by progenitor com-

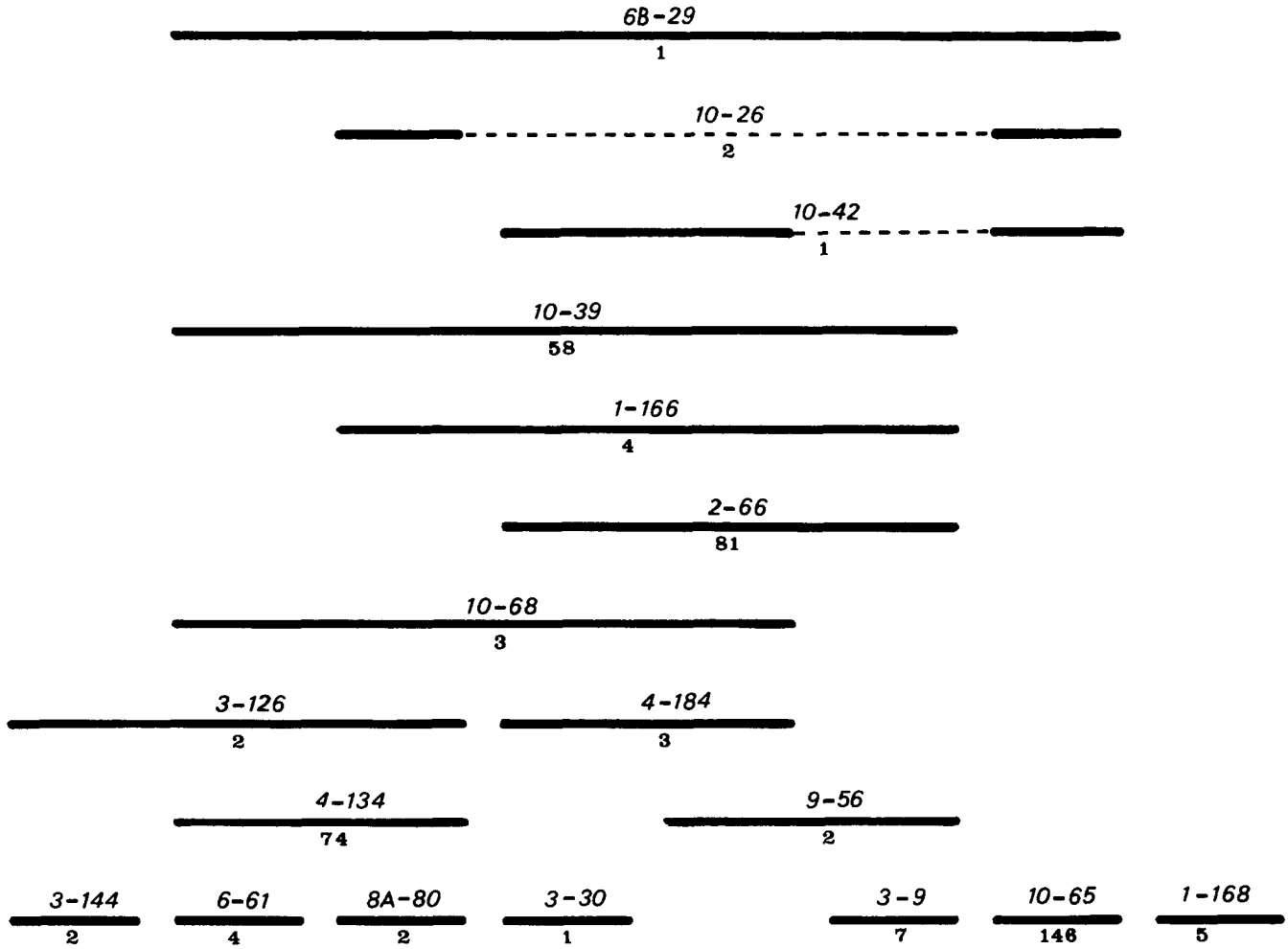

FIGURE 3.—Accumulated complementation map of putative deficiencies carried by detachment products from all 12 detachment experiments. Each bar represents a different complementation group. Bars that do not overlap represent complementing groups, and vice versa. The number above each bar designates the detachment product used to represent the complementation group. The number below the bar indicates the number of detachment products assigned to the complementation group. Dotted lines represent discontinuous deficiencies. See text for further details.

pound chromosomes carrying a deficiency. The 68% frequency of lethals among experiment 10 detachments is consistent with this. It should be noted that, to this point, the arrangement of the complementation map drawn in Figure 3 is to some degree arbitrary. Other variations of this map are also consistent with the results. However, these ambiguities will be resolved below.

In addition to the detachment chromosomes shown on the complementation map, 44 chromosomes behaved as random hits, that is, mutational events other than those involved in generating the detachment. These chromosomes complemented all others against which they were tested. Twenty of the putative random hit lethals from different experiments were selected and complementation tested in all *inter se* combinations. All crosses resulted in complementation, providing further evidence that during the detachment event these chromosomes received random, third hits responsible for generating lethals elsewhere on the chromosome.

One additional detachment product included in the complementation analysis, but not shown on the complementation map, was lethal *11–64*. This detachment chromosome was semilethal over all deficiencies of the region defined by lethal *3–30*, and surviving heterozygotes had slightly outspread wings with only a partial posterior cross-vein. The *11–64* chromosome fully complemented detachment products from all other complementation groups. Unfortunately, this stock was lost, making further tests impossible.

Vials containing detachment products of the *3–9* complementation class also exhibited a phenotype. Rare homozygous adults eclosed, but died within 48 hr of eclosion. Surviving adults had a phenotype resembling *rotund*, with droopy wings and reduced sex combs in males. Nonetheless, all detachment products of this class fully complemented *rotund*, which has

been mapped to band 84D on the right arm of chromosome *3* (DUNCAN and KAUFMAN 1975). The phenotype expressed by homozygotes of the *3–9* complementation group was designated *rotund-like*.

A number of nonlethal detachment products were also tested for complementation with some of the lethal complementation groups. If the heterochromatin of chromosome *3* contains tandemly repeated genes, it is possible that partial deletions would be homozygous viable. However, partial deletions may be lethal over more severe deficiencies. To test this possibility, approximately 150 nonlethal detachment products were each crossed to lethals *3–126*, *1–166* and *10–65*, which collectively span the accumulated complementation map. However, all the crosses resulted in full complementation, and the non-lethal detachment products were discarded. As well, to keep stocks manageable, a maximum of ten lethal lines continued to be maintained for each complementation group.

Recombination mapping: The proximal position of the putative heterochromatin deficiencies was confirmed using the recombination mapping protocol described in the MATERIALS AND METHODS section. Exchange events between tightly linked proximal markers on either the left or right arm were recovered from females heterozygous for a detachment product and a marker chromosome. For most reconstituted thirds, the lethal deficiency always remained with the recombinant product containing the centromeric fragment of the detachment chromosome. This was the case for detachment products, *10–65*, *10–42*, *4–134*, *10–39*, *6B–29*, *3–9*, *6–61*, and *4–75*. These deficiencies, which include most of the complementation groups, are therefore definitely located in the proximal region of chromosome *3*.

The lethals of detachment products *1–168* and *3–144* (Figure 3), however, mapped to the right arm euchromatin. The complementation group represented by putative deficiency *1–168* consists of five lethal detachments, all recovered in experiment 1. The lethals in this group complement all other complementation groups. It should be noted that all 12 compound strains used in this study had been maintained in stock for close to a year prior to detachment. It seems likely that a spontaneous recessive lethal arose in the right-arm euchromatin and spread within a subpopulation of the experiment 1 compound stock. Consequently, approximately 50% of the detachment products generated from females bearing the lethal mutation would also carry the lethal. This explanation is consistent with the finding that spontaneous recessive lethals do accumulate on compound chromsomes (PARKER 1954).

A similar explanation is likely for lethal *3–144*. The three other chromosomes that do not complement *3–144* are all from experiment 3. Two of these lethals, from the *3–126* complementation group, also failed to complement many other deficiencies in the proximal region. Recombinational analysis of detachment-product *3–126* disclosed two separable lethal sites on this chromosome, one in the proximal region and one in the right euchromatin that failed to complement *3–144*. Therefore, it would appear that a spontaneous lethal arose in the right arm euchromatin of a subpopulation of the *C(3R)* chromosome used in experiment *3*. Of the four detachment products carrying this lethal, two also carried a proximal deficiency that arose during the detachment event.

Lethals on four of the 44 detachment chromosomes initially classified as random hits were also located through recombinational analysis. Three of the four lethals mapped outside the proximal region, but the fourth, designated *10–33*, mapped to the proximal region. This chromosome was tested against all remaining lethal detachment products in complementation groups that possibly extended further than what had been revealed by the complementation map. Three of the ten strains from the *10–39* complementation group and five of the ten from the *4–134* group failed to complement *10–33*. Therefore, *10–33* defines a new complementation group that was previously undetected because the tester chromosomes used to represent the *10–39* and *4–134* complementation groups did not include *10–33*. Consequently, both the *10–39* and the *4–134* groups must be redefined as containing at least two subgroups, one that includes the *10–33* region and one that does not. In view of this finding, mapping studies were conducted on an additional ten chromosomes whose lethals had been classified as random hits. However, the results were in agreement with the original classification as no additional complementation groups were disclosed.

Reattachment of detachment products: Deficiencies associated with detachment products were assigned either to the left or to the right arm heterochromatin from the results of generating compound autosomes from the detachment chromosomes. Females heterozygous for *TM3* and one of the detachment chromosomes, selected from the various complementation groups, were irradiated and mated to males carrying compound thirds. For each deficiency-bearing chromosome, 2500 females were treated with 2500 rad and an additional 1250 females were treated with 4000 rad of γ-radiation. In addition to those offspring arising from nondisjunctional events, individuals were recovered with a newly synthesized sister-strand compound-*3* chromosome. Since sister-strand attachments are homozygous for one of the chromosomal arms of the lethal-bearing detachment chromosome, only *C(3L)*'s should be rescued from detach-

TABLE 3

Sister-strand compound thirds recovered from the radiation-induced reattachment of selected detachment products

Detachment product	Sister-strand compound recovered[a]	
	C(3L)	*C(3R)*
3-9	0	5
3-30	0	6
1-16	0	2
1-166	0	4
2-66	0	2
10-26	0	0
10-42	0	0
10-65[b]	5	1
10-65[b]	4	0
1-168	21	0
ri p[pc]	6	8

[a] A total of 3750 heterozygous females were treated for each reattachment test. See text for details.

[b] This experiment was repeated owing to the unexpected recovery of a *C(3R)* chromosome in the first reattachment test.

[c] As a control sister-chromatid attachments were generated from the isogenic *ri p*[p] chromosome balanced over *TM3*.

ment products carrying a deficiency of the right arm, and vice versa. The results of the reattachment experiments are summarized in Table 3. Only new *C(3R)*'s were generated from tester chromosomes representing the *3-9*, *3-30*, *1-16*, *1-166* and *2-66* complementation groups. Therefore, these complementation groups and others that fail to complement them are presumed to represent deficiencies of the left arm heterochromatin. As expected, the *10-26* and *10-42* chromosomes failed to produce any new sister-strand attachments of the left or right arm, confirming that these detachment chromosomes carry double deficiencies.

The *10-65* chromosome reattachment experiment was repeated because, in the first experiment, five new sister-strand *C(3L)*'s and one new *C(3R)* were recovered. The recovery of compounds from both arms would have suggested that the *10-65* chromosome carried a lethal on neither arm, which is clearly not the case. To resolve this anomaly, the *10-65* reattachment experiment was repeated, and this time only new *C(3L)*'s were recovered. It is evident that the *10-65* chromosome does carry a lethal on the right arm. The single *C(3R)* recovered in the first experiment remains unexplained.

Reattachment results with the *1-168* chromosome are also peculiar. As explained above, this chromosome carries a lethal outside the proximal region somewhere in the right euchromatin and this is confirmed by the recovery of only *C(3L)* chromosomes. However, the relatively large recovery of reattachments is difficult to explain. It appears that this detachment chromosome differs significantly from the others tested in its ability to undergo further rearrangements. A similar finding had been observed in the original detachment experiment, where one particular compound chromosome exhibited a significantly reduced propensity to be detached. These results suggest that third chromosomes previously involved in attachment-detachment events with heterochromatic breakpoints differ widely in their competency to be involved in further rearrangements.

A control reattachment experiment was conducted by reattaching the isogenic *ri p*[p] chromosome while balanced over *TM3*. Since the *ri p*[p] chromosome does not carry any recessive lethals, it should produce new sister-strand attachments of both the left and right arms. As predicted, both *C(3L)*'s and *C(3R)*'s were recovered in approximately equal numbers.

Further analysis of compound and detachment chromosomes: Figure 4 contains the revised complementation map that includes the results of the recombination mapping and reattachment experiments. This map suggests the presence of six proximal loci on the left arm and one on the right. The right arm locus is designated *l(3)Rh1*, and the six lethal loci on the left arm are numbered *l(3)Lh1* to *l(3)Lh6*, where the *h* designates heterochromatin. Although the complementation map is shown with *l(3)Lh1* proximal and *l(3)Lh6* distal on the left arm, which is the simplest interpretation, the results to this point cannot distinguish between this possibility and the opposite orientation. However, the orientation of the deficiencies on the left arm can be determined by genetically characterizing the original compounds used in the detachment experiments, since deficiencies or duplications carried by the compounds will be polar (*i.e.*, they will include the most proximal gene and extend distally). Individual complementation maps for each of the twelve detachment experiments were produced from the overall map, and then analyzed for clues about the genetic makeup of the compounds that were progenitors.

If an ancestral compound carried a deficiency, over 50% of all detachment-products from that compound would carry a deficiency for the same specific region. Analysis of the data revealed that over 50% of the detachment chromosomes produced in all four experiments involving *C(3R)VM3* carried deficiencies of *l(3)Rh1*. Therefore, *C(3R)VM3* was heterozygous for a deficiency of *l(3)Rh1*. Similarly, two of three experiments using *C(3L)VM3* produced over 50% lethals for a specific region on the left arm, indicating that *C(3L)VM3* carried a deficiency of left arm heterochromatin. The third experiment involving this compound, which produced fewer than 50% lethals for any region, must have involved a *C(3R)* carrying a duplication that compensated for the deficiency carried on *C(3L)VM3*. The centromere-spanning deficiencies produced in experiment 10, where *C(3R)VM3*

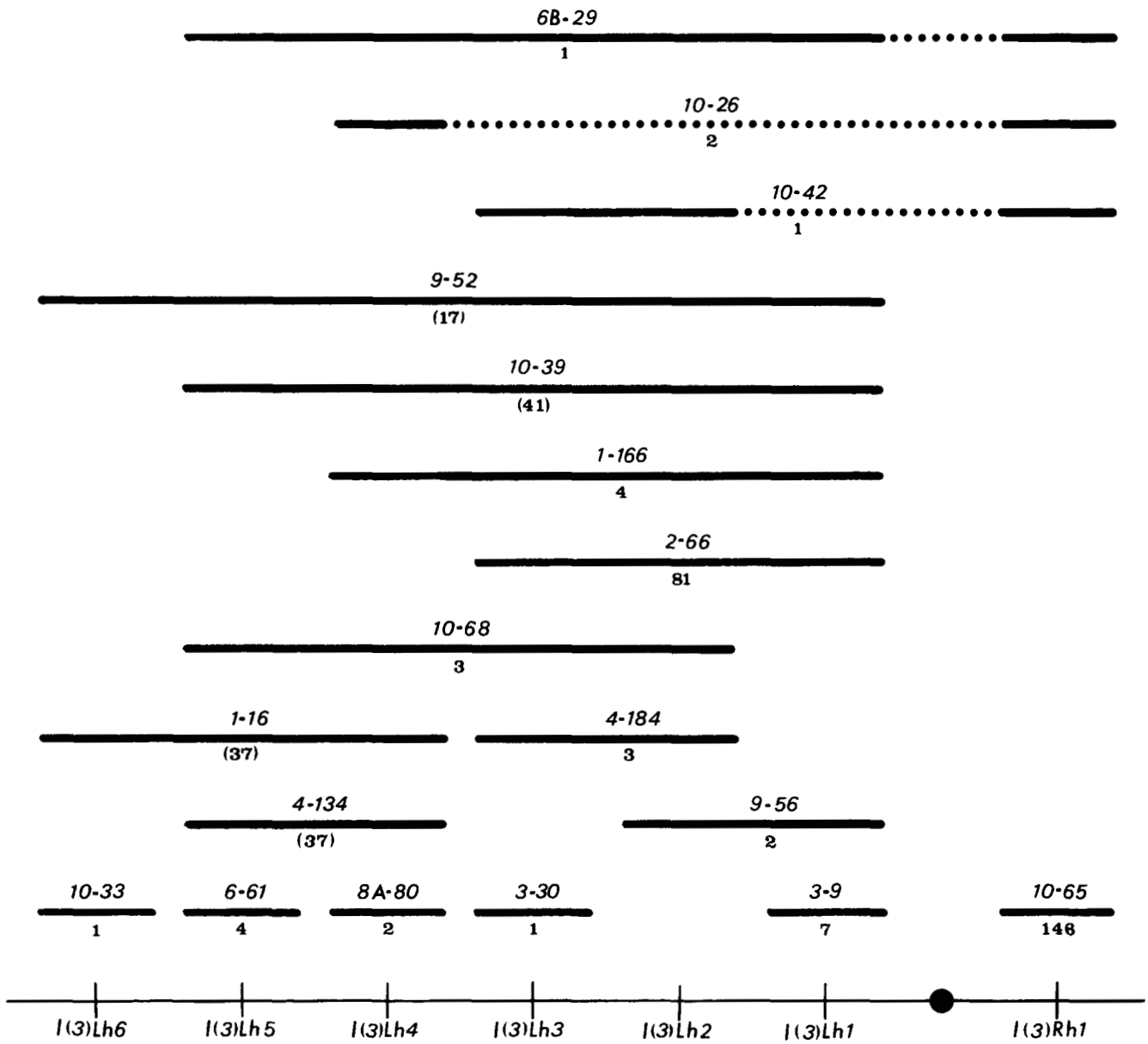

FIGURE 4.—Revised complementation map incorporating results of recombination mapping and reattachment experiments. The number above each bar designates the detachment product used to represent each complementation group; and the number below the bar indicates the number of detachment products assigned to each group. The number of detachment products in the *4-134*, *1-16*, *10-39*, and *9-52* complementation groups is shown in brackets because they are estimates. Estimates were necessary because only some of the detachment products in these groups were available for complementation tests with the previously undetected *10-33* group.

was paired with *C(3L)VM3*, were diagnostic of deficiency-carrying compounds and confirmed that at least one of the two compounds carried a deficiency. Moreover, from experiment 6, the centromere-spanning deficiency places the break in (C3L)*VM3* to the region separating *l(3)Lh5* and *l(3)Lh6*. Deficiencies were not associated with any of the remaining progenitor compound autosomes.

Any detachment experiment that does not involve a deficiency-carrying compound, but which results in nonpolar detachment deficiencies must involve a progenitor compound carrying a duplication. However, it is not possible to identify all nonpolar deficiencies without first knowing the proximal-distal orientation of the deficiencies. To solve both unknowns simultaneously, a set of possible models of the progenitor compound chromosomes was constructed and analyzed to determine if it could account for all the detachment-product classes generated in the 12 experiments. The only configuration that could explain all 443 detachment products had the orientation of the left arm as shown in Figure 4 and the progenitor compounds described in Table 4. Using this set of progenitor compounds and orientation of the left arm, possible breakpoints were identified for every detachment chromosome.

TABLE 4

Duplications and deficiencies carried by progenitor compound-3 chromosomes as predicted from the analysis of detachment products

Progenitor	Duplication	Deficiency
C(3L)VM1	None	None
C(3L)VM2	None	None
C(3L)VM3	None	*l(3)Lh1-5*
C(3L)VM4	None	None
C(3R)VM1	*l(3)Lh1-3*	None
C(3R)VM2	None	None
C(3R)VM3	*l(3)Lh1-6*	*l(3)Rh1*

Two ambiguities remained for the compound chromosome configurations described in Table 4. First, the duplication carried by *C(3R)VM3* could extend to *l(3)Lh5* or *l(3)Lh6*. Both possibilities were consistent with the data, and could not be distinguished further. The second ambiguity arose from the uncertainty of whether any of the four *C(3L)* chromosomes carried duplications. With only one complementation group on the right arm, it was not possible to identify nonpolar deficiencies that would be diagnostic for a duplication carried on *C(3L)*. This ambiguity was resolved by a reattachment experiment which tested the capability of progenitor compounds to rescue homozygous deficient sister-strand attachments. As described above, no *C(3R)* sister-strand attachment will be recovered from a detachment product carrying a deficiency on the right arm, and vice versa. However, when females carrying a detachment product with a deficiency on the right arm balanced over *TM3* are irradiated and mated to males carrying a *C(3L)* with a duplication of the right arm, the duplication should be able to rescue homozygous deficient sister-strand *C(3R)*'s.

Each of the seven progenitor compounds used in the detachment experiments was tested for the presence of duplications by this method. The results of the reattachment experiments are shown in Table 5. Each of the four *C(3L)* chromosomes was tested by mating 8000 irradiated females, heterozygous for the right arm deficiency *10-65*, to males carrying the *C(3L)VM* chromosome being tested. Offspring were screened for the presence of newly synthesized *C(3R)*'s. Since no sister-strand *C(3R)*'s were recovered, the four progenitor *C(3L)* chromosomes must not carry duplications of the region defined by the *10-65* deficiency.

The three *C*(3R)*VM* chromosomes were tested in a similar manner. Females heterozygous for the left arm deficiency *3-9* (Figure 4) were treated with 4000 rads and mated to males carrying a progenitor *C(3R)VM* chromosome. Newly synthesized sister-strand *C(3L)*'s were rescued by *C(3R)VM1* and *C(3R)VM3*, but not by *C(3R)VM2*. These results confirmed that

TABLE 5

Rescue of homozygous deficient sister-strand compound-3 chromosomes by progenitor compounds[a]

Progenitor compound	Deficiency chromosome tested	Predicted duplications	Homozygous deficient compound-3's rescued	
			C(3R)	*C(3L)*
C(3L)VM1	*10-65*	None	0	
C(3L)VM2	*10-65*	None	0	
C(3L)VM3	*10-65*	None	0	
C(3L)VM4	*10-65*	None	0	
C(3R)VM1	*3-9*	*l(3)Lh1-3*		5
C(3R)VM2	*3-9*	None		0
C(3R)VM3	*3-9*	*l(3)Lh1-6*		6

[a] In each of the seven experiments, 8000 radiation-treated females were used.

C(3R)VM1 and *C(3R)VM3* were the only progenitor compounds carrying duplications of vital loci on proximal *3L* and supported the polarity of the map presented in Figure 4.

Distribution of putative heterochromatic loci: The breakpoints involved in the formation of the complete set of detachment products are not evenly distributed along the complementation map. Some regions of the map have a high frequency of breakpoints whereas breakpoints in other regions are rare. If the distance between two genes is assumed to be proportional to the number of breakpoints that occur in that region, it is possible to construct a distribution map of the genes in the third chromosome heterochromatin (Figure 5).

One difficulty in producing such a map was that many experiments involving a progenitor compound carrying a duplication or deficiency provided two or more possible sets of breakpoints for generating the same detachment chromosome. Therefore, when calculating the map distances for either the left or right arm, experiments that involved compounds carrying a deficiency or duplication for the arm being examined were excluded from the analysis. As a result, the only experiments used in the calculations were those where the breakpoints could be assigned to a specific region with complete certainty.

For the right arm, four experiments involved compounds with a deficiency of the right arm and thus had to be excluded from the analysis. In the remaining eight experiments, only 22 of a total of 649 detachment products were deficient for the gene(s) defined by the *10-65* deficiency. If a breakpoint occurs on the right arm proximal to the *10-65* region, none of the resulting detachment-products will carry a deficiency for this region. If, on the other hand, the breakpoint occurs distal to the *10-65* region, then only one-half of all detachment products will be deficient for this region. That is, assuming an equal probability of

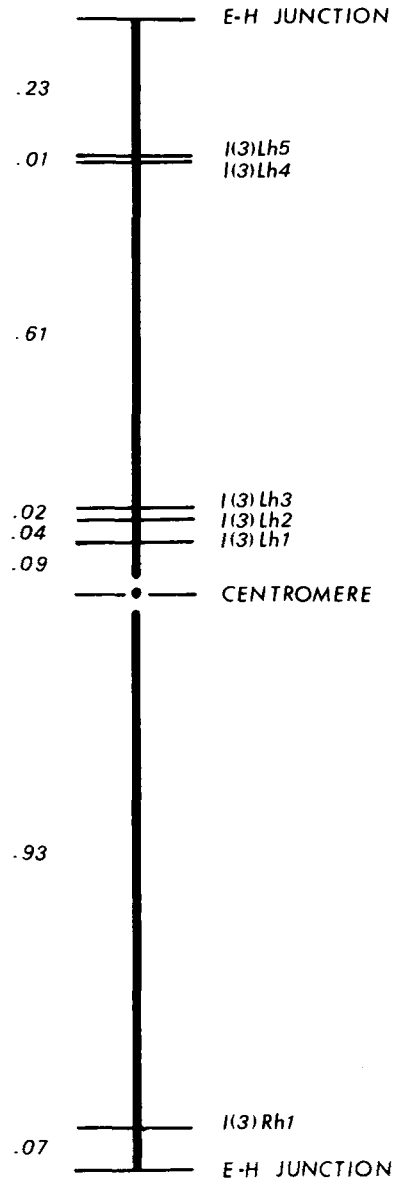

FIGURE 5.—Distribution of genes in the proximal heterochromatin of chromosome *3*. Relative map distances are based on the number of radiation breakpoints induced in each region. See text for details.

recovering either the centric or acentric fragment, one of the two detachment products will carry a duplication of the *10-65* region, the reciprocal detachment-product will carry a deficiency. Therefore, the actual frequency of breakpoints that occur distal to a particular locus is twice the frequency of deficiencies recovered for that locus. If it is assumed also that detachment-product breakpoints occur only in heterochromatin and are randomly distributed within the heterochromatic block, then the distance of the *10-65* region relative to the centromere from the euchromatic-heterochromatic junction is calculated as follows:

2 (No. of detachments deficient for *10-65*/total No. of detachments) × 100% = 2 (22)/649 × 100% = 7%.

Therefore, on the basis of breakpoint frequencies, the *10-65* region is located at a position 7% of the distance from the euchromatic-heterochromatic junction to the centromere.

Similar calculations were made for each locus on the left arm. Only data from experiments 2, 5 and 9 were used, since these were the only experiments not involving compounds carrying duplications or deficiencies of the left arm. The relative positions of *l(3)Lh1*, *l(3)Lh2*, *l(3)Lh3*, *l(3)Lh4*, and *l(3)Lh5* were 91%, 87%, 85%, 24% and 23% respectively of the distance from the left euchromatic-heterochromatic junction to the centromere. The map position of *l(3)Lh6* was not calculated owing to the extremely low number of detachment products tested for a deficiency of this site. However, a very rough estimate would place it half-way between *l(3)Lh5* and the left euchromatic-heterochromatic junction.

The map distances shown in Figure 5 are proportional to the number of radiation breakpoints induced in each region. However, these map distances are proportional to the actual physical distances between loci if and only if radiation breakpoints are randomly distributed within the heterochromatic block.

Lethal phases: The lethal phases for several of the detachment-product deficiencies were determined using egg count experiments. Females carrying a deficiency over *TM3* were mated to sibling males and then allowed to lay eggs for three successive two-hour periods on petri dishes containing fresh medium. The final petri dish in each series was examined daily. All deficiencies tested had larval lethal phases during either the second or third instar stages.

DISCUSSION

In the experiments described above, a series of compound-autosome attachments, detachments and reattachments were generated and used to dissect the heterochromatin of chromosome *3* of *D. melanogaster*. The analysis of approximately 400 proximal deficiencies carried by detachment products, revealed the presence of at least seven vital genetic loci in the proximal region of chromosome *3*. Attempts to reattach chromosomes carrying deficiencies of these genes established that six of the genes were on the left arm and that one was on the right. This study has further demonstrated the usefulness of compound-autosome attachment and detachment procedures for analyzing the proximal regions of Drosophila autosomes by providing a genetic map of what has been until now the largest uncharted region of the *D. melanogaster* genome.

Several modifications of the compound attachment

and detachment procedures were used in the course of this study. First, reattachment of deficiency-carrying detachment products identified the position of deficiencies relative to the centromere. Second, the ability of compound autosomes to rescue newly synthesized, homozygous-deficient, sister-strand attachments was used to test for the presence of duplications on compound chromosomes. Finally, the use of many different combinations of compound chromosomes in detachment experiments permitted the genetic characterization of the progenitor compounds and, therefore, a detailed and useful breakpoint analysis of all the resulting detachment products.

Large-scale production of detachment products generated from various combinations of compound autosomes, heterozygous for deficiencies and (or) duplications of the proximal heterochromatin, permitted the recovery of five complementing deficiencies on the left arm, and, through overlapping deficiencies, the recognition of six complementing regions. In the absence of preexisting duplications or deficiencies on the compounds, known visible genetic markers or semilethality, as found by HILLIKER and HOLM (1975) for chromosome *2* heterochromatin, all deficiencies would be polar, and consequently overlapping. For the segments that lie distal to region 6 on the left arm and region 1 on the right, no further resolution is possible by the detachment technique without corresponding duplications or preexisting deficiencies. The absence of resolution within the right heterochromatin suggests that possibly only one genetic locus resides in this region and the low frequency with which it is uncovered through detachments implies that it occupies a distal site in the right heterochromatin. Genes proximal to the most proximal complementation group on either side, but mainly to the right, of the centromere, may not have been detected if they were tandem repeats. However, after testing 150 nonlethal detachments against the largest deficiencies that collectively span the complementation map, we found no evidence to support the notion of tandemly repeated vital loci within the deleted segments of heterochromatin.

There is convincing evidence that the genes uncovered by detachment-product deficiencies lie within heterochromatin. HILLIKER and HOLM (1975) found deficiencies on chromosome *2* to be genetically proximal to previously isolated deletions that extended from the euchromatin into the distal heterochromatin. Similarly, the deficiencies of the third chromosome recovered by BALDWIN and SUZUKI (1971) complemented all known proximal euchromatic markers on the third chromosome. Cytological observations of polytene chromosomes carrying detachment-product deficiencies of chromosome *2* did not detect removal of any euchromatin (HILLIKER and HOLM 1975), and cytological and genetic analysis of numerous compound-*2* chromosomes, even those carrying duplications for the *rl* or *lt* genes, failed to disclose duplications for euchromatic loci (HOLM 1976). The results of the present study are also consistent with a heterochromatic location for vital genes uncovered by detachment-product deficiencies. Examinations of polytene chromosomes from larvae carrying detached thirds revealed no obvious duplications or deficiencies of the proximal euchromatic bands, and observations of metaphase preparations of three detachment products (MARCHANT and HOLM 1988) verified that genetic deletions of the more proximal loci correspond to cytological deficiencies restricted to the heterochromatic blocks.

The genetic map presented in Figure 5 for the relative position of vital loci in the proximal heterochromatin of chromosome *3* portrays a very uneven distribution of breakpoints along the complementation map. If we assume that the physical distance between any two genes is proportional to the number of radiation induced breakpoints that occur in that region, and that all regions of the heterochromatin are equally susceptible to radiation-induced interchange, then, as shown in Figure 5, the genes to the left of the centromere occur in clusters throughout the heterochromatin. The single identifiable locus to the right of the centromere is close to the heterochromatic-euchromatic boundary.

However, from other studies, there is some limited experimental evidence that radiation-induced breakpoints are not evenly distributed within heterochromatin. Visual examinations of a series of radiation-induced rearrangements with heterochromatic breakpoints detected a preponderance of interchanges at or near the euchromatic-heterochromatic junction (GATTI, TANZARELLA and OLIVEIRI 1974; SCHUBERT and RIEGER 1976). KENNISON (1981) also observed a striking nonrandom distribution of translocation breakpoints on the heterochromatic *Y* chromosome, with most if not all breakpoints within nonfluorescent segments, or near junctions between differentially staining regions. If radiation breakpoints in heterochromatin are nonrandomly distributed and possibly clustered near the euchromatic-heterochromatic junction, then the radiation breakpoint map in Figure 5 is skewed toward the centromere, and the real positions of the genes are more distal than shown. Extensive cytological analysis of heterochromatic deficiencies generated in standard chromosomes will be required to disclose the exact location of these genetic loci.

In connection with the generation of rearrangements and the distribution of breakpoints in the heterochromatin, two other findings merit attention. The first is that attached and detached chromosomes can differ widely in their propensity to undergo further

rearrangements. In the detachment of compound autosomes, all experiments involving *C(3R)VM2* consistently generated significantly fewer detachment products than experiments involving the other compound autosomes. Similarly, the *3-126* detachment chromosome was several times more likely to undergo reattachment than the other detachment products tested.

The second observation of possible significance is that detachment products carrying a large deficiency tend to carry a compensating duplication. Three detachment products from this study that were analyzed by S. PIMPINELLI (personal communication), to be discussed in more detail in the next paper (MARCHANT and HOLM 1988), contained relatively large deficiencies compensated by large duplications. As a result, there was an approximately normal-sized heterochromatic block on both sides of the centromere of each reconstituted, standard, third chromosome. HILLIKER and HOLM (1975) observed a similar phenomenon with second chromosome heterochromatic deletions. Although their reconstituted second chromosomes carried genetically large deficiencies, the mitotic chromosomes appeared normal, suggesting that the deletions were compensated by accompanying duplications. Additionally, from the genetic results of this study, the deficiency of region 1 in the right heterochromatin of *C(3R)VM3* was compensated by a duplication of regions 1 to 6 from the left heterochromatic block. It seems unlikely that chromosomes with little or no heterochromatin on one arm cannot be rescued, as *Df(2R)MS2-10* (MORGAN, SCHULTZ and CURRY 1940), even though not a detachment product, carries a cytologically visible deficiency of most, if not all, right arm heterochromatin (HILLIKER and HOLM 1975). Therefore, the apparent conservation of heterochromatic symmetry flanking the centromere suggests something intrinsic about the process of attaching and detaching compound autosomes.

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