Spontaneous Formation of Compound *X* Chromosomes in *Drosophila melanogaster*

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ABSTRACT

Males carrying different *X* chromosomes were tested for the ability to produce daughters with attached-*X* chromosomes. This ability is characteristic of males carrying an *X* chromosome derived from *59b-z*, a multiply marked *X* chromosome, and is especially pronounced in males carrying the unstable *59b-z* chromosomes *Uc* and *Uc-l^r*. Recombination experiments with one of the *Uc-l^r* chromosomes showed that the formation of compound chromosomes depends on two widely separated segments. One of these is proximal to the *forked* locus and is probably proximal to the *carnation* locus. This segment may contain the actual site of chromosome attachment. The other essential segment lies between the *crossveinless* and *vermilion* loci and may contain multiple factors that influence the attachment process.

THE first compound chromosomes in Drosophila were discovered by L. V. MORGAN (1922). The case she described involved the spontaneous attachment of two *X* chromosomes, probably by crossing-over in the proximal heterochromatin. This process leads to the formation of reversed metacentric compound *X* chromosomes. If one exchange point is in the heterochromatin of the short arm of one *X* and the other is in the heterochromatin of the long arm of the other, the resulting metacentric chromosome has a single active centromere and is meiotically and mitotically stable. Other instances of such *X* chromosome attachment have been encountered since Morgan's discovery (LINDSLEY and GRELL 1968). In addition, other types of attached chromosomes have been detected, including cases involving the attachment of autosome arms (NOVITSKI 1963; LINDSLEY and SANDLER 1963). Most of these cases have resulted from recombination between rearranged chromosomes or from X-ray-induced attachment.

There does not appear to have been any systematic attempt to study the spontaneous formation of attached chromosomes, possibly because attachment is a rare event. Here we report on the formation of reversed metacentric compound *X* chromosomes in strains that produce these rather frequently. These strains carry either a multiply marked *X* chromosome called *59b-z* or a derivative of that chromosome. The frequency of attachment is especially high in lines that carry the *Uc* or *Uc-l^r* derivatives (LIM 1981a). These latter chromosomes have been studied for their unusual mutability and also for their tendency to accumulate structural rearrangements (LIM 1979, 1981b; LAVERTY and LIM 1982, LIM *et al.* 1983). On account of these properties, the *Uc* and *Uc-l^r* chromosomes are referred to as unstable *X* chromosomes.

The high frequency of attached-*X* chromosome formation in the unstable *X* chromosome stocks has made it possible to analyze the attachment process genetically. The experiments described here establish that this process involves two widely separated segments on these chromosomes, one evidently containing the attachment site, the other containing factors that influence the attachment process.

MATERIALS AND METHODS

Stocks: All stocks and experimental cultures were raised on a standard cornmeal-molasses medium at 25°. For detailed information on the genetic markers, see LINDSLEY and GRELL (1968).

1. *59b-z* = *y^{59b} z w^i ct^6 f*, a stock homozygous for five recessive markers on the *X* chromosome. The map positions and phenotypes of these are 0.0 (*y* = yellow body), 1.0 (*z* = zeste eyes), 1.5 (*w^i* = white-ivory eyes), 20.0 (*ct* = cut wings), and 56.7 (*f* = forked bristles). The *w^i* allele is epistatic to *z*.

2. *C(1)DX, y f/Uc/Y*, a stock in which the females have attached-*X* chromosomes homozygous for *y* and *f* and the males have an unstable *X* chromosome derived from *59b-z* (LIM 1979, 1981b). This latter chromosome has the same markers as *59b-z* but is difficult to maintain in homozygous condition due to female sterility.

3. *C(1)DX, y f/Uc-l^r/Y*, a set of stocks in which the *X* chromosome of the male was derived from *Uc* through the reversion of a recessive lethal mutation that had occurred on it (LAVERTY and LIM 1982). Different *Uc-l^r* chromosomes are distinguished by different identity numbers; all of the *Uc-l^r* chromosomes used here were derived independently from a set of sibling lethal lines that came from a single

TABLE 1

Formation of attached-X chromosomes in males carrying various *Uc-l*^{r} chromosomes

Uc-l^{r} chromosomes	No. males tested	No. males with ≥1 exceptional daughter	No. sons	No. exceptional daughters	Percent exceptional daughters ± SE
10.1	9	1	484	1	0.20 ± 0.20
10.2	8	0	378	0	0
10.4	9	0	372	0	0
10.5	9	6	437	31	7.00 ± 3.00
15.21	10	9	472	59	11.08 ± 2.74
15.22	10	3	392	4	1.22 ± 0.67
15.23	10	2	430	4	1.93 ± 1.75
15.24	8	0	384	0	0
16.41	8	6	271	20	7.69 ± 2.13
16.42	9	7	393	15	3.67 ± 0.96
16.43	7	2	303	3	0.81 ± 0.57
16.44	9	5	348	13	3.44 ± 1.50
17.17a	7	0	393	0	0
17.17b	10	0	611	0	0
17.17c	8	0	399	0	0
17.17d	10	1	599	1	0.16 ± 0.16
19.19a	9	3	448	4	0.93 ± 0.51
19.19b	7	3	490	7	1.68 ± 0.86
19.19c	10	3	872	4	0.59 ± 0.33
19.19d	9	3	752	4	0.57 ± 0.29

Flies were counted until the 19th day after mating. The frequency of exceptional daughters is the unweighted average of the cultures that were scored.

Uc male. The lethals in this cluster were allelic with each other and presumably originated in a single premeiotic mutational event.

4. *C(1)DX, y f/y cin w f*^{s} *su(f)*^{ts67g}*/Y*, an attached-*X* stock in which the males have a temperature-senstive lethal mutation. This mutation facilitates the collection of large numbers of attached-*X* virgin females (SIMMONS *et al.* 1980).

5. *m*, a stock homozygous for an *X*-linked recessive mutation causing miniature wings (map position 36.1).

6. *cv v car*, a stock homozygous for three *X*-linked recessive markers, with map positions 13.7 (*cv* = crossveinless wings), 33.0 (*v* = vermilion eyes) and 62.5 (*car* = carnation eyes). Flies with the combination *v car* can be distinguished from flies with either mutation alone.

7. *C(1)DX, y f/FM6, y*^{31d} *sc*^{8} *dm B/ Y*, an attached-*X* stock in which the males have the *FM6* balancer *X* chromosome containing recombination-suppressing inversions and the semidominant marker, *B* (Bar eyes).

8. *FM7, y*^{31d} *sc*^{8} *sn*^{x2} *B/ Ins(1)sc*^{7} + *AM, sc*^{7} *w*^{a} *ptg*^{4} *Bx*^{78h} *1(1)*^{78h}, abbreviated *FM7/sc*^{7} *1*, a stock with the *FM7* balancer *X* chromosome which, like *FM6*, carries recombination-suppressing inversions and the semidominant marker *B*.

9. Canton S, a wild-type stock.

Detection of newly formed attached-X chromosomes. Newly formed attached-*X* chromosomes were detected by crossing *X/Y* males individually with 2–3 *C(1)DX, y f/Y* females from stock 4. Exceptional females, phenotypically like their fathers, were tested for attached-*X* chromosomes by mating them individually to *FM6* or *FM7* males. The appearance of only Bar sons and non-Bar daughters was taken as evidence that *X* chromosome attachment had occurred.

Cytological analysis of attached-X chromosomes in larval ganglion squashes. Early third instar larvae were dissected in a drop of Shen's solution (0.25 M NaCl, 5.6 mM KCl and 0.5 mM CaCl) on a siliconized slide. The ventral ganglion and brain hemisphere were removed and transferred into Hank's essential medium with 1 μg/ml Colcemid. The tissues were kept in this solution for 1.5–2 hr. and then transferred to a hypotonic solution of 75 mM KCl for 5–10 min. The ganglion and brain were then fixed in a drop of a 1:1 mixture of acetic acid and methyl alcohol for 3–5 min. A drop of acetic orecin was added to stain the preparation and a coverslip was gently placed on top. The slides were then examined with a phase contrast microscope.

RESULTS

Frequency of attachment of X chromosomes from various stocks: The phenomenon of *X* chromosome attachment was discovered in experiments with *Uc-l*^{r} stocks. The males in each of these stocks carried a nonlethal *X* chromosome which had been derived from a lethal *Uc* chromosome by reversion of the lethal mutation. These lethal chromosomes all traced back to a single *Uc* male that had produced a cluster of lethal *X* chromosomes in its progeny. Each of the lethal mutations in this cluster was shown to map in bands 6F1-2 of the polytene *X* chromosome (LIM 1979; LAVERTY and LIM 1982), indicating that the members of the cluster were almost certainly derived from the same premeiotic mutational event. Altogether, 20 *Uc-l*^{r} stocks were tested for *X* chromosome attachment, each representing an independent reversion of the primary lethal mutation.

The results of these tests are given in Table 1. A total of 176 males from the 20 stocks were tested. These produced 9228 sons and 170 exceptional

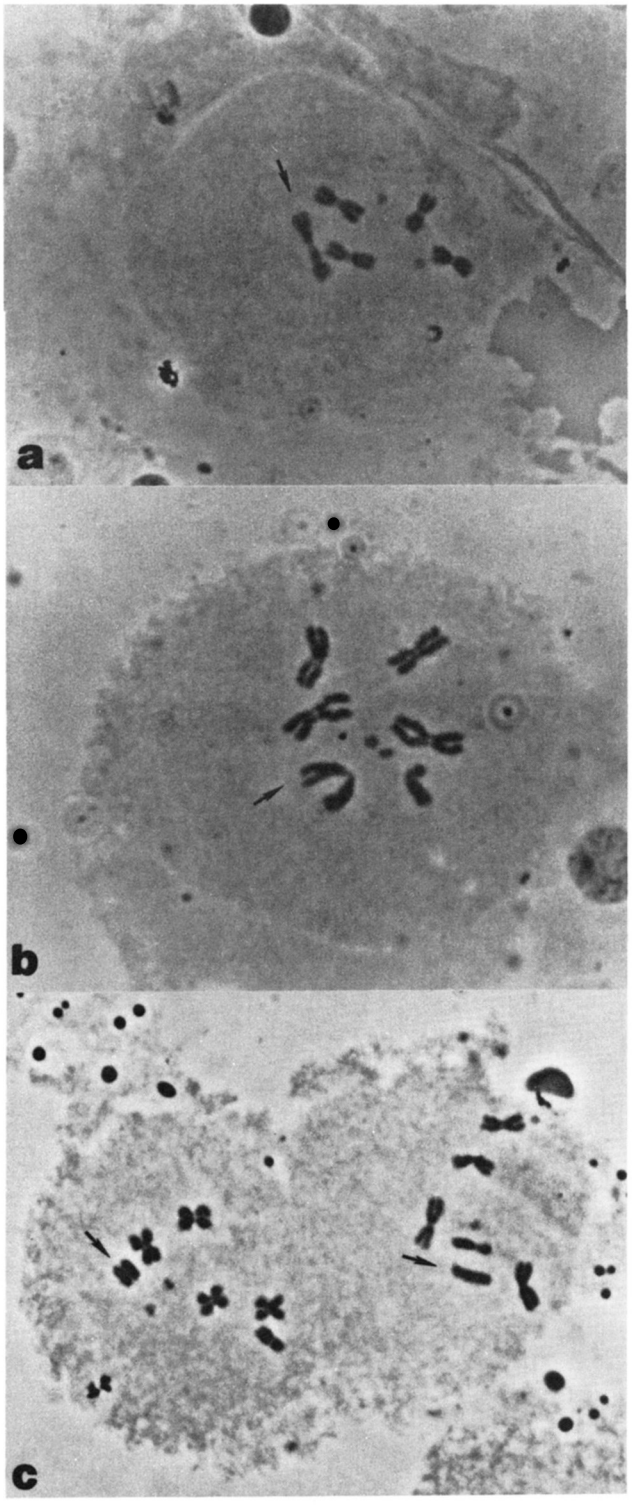

FIGURE 1.—Larval ganglion squashes showing metaphase nuclei with attached-*X* chromosomes. In each nucleus, the attached-*X* chromosomes are indicated by an *arrow*. (a and b) Attached-*X* chromosomes derived from *Uc-l*^{r}. The metaphase spread in (a) lacks a *Y* chromosome, whereas the spread in (b) has this chromosome. In both photographs, note that heterochromatin flanks the centromere on both sides. (c) *C(1)DX, y f*, the classic double-*X* chromosome of MULLER (1943).

daughters, the latter having the same phenotype as their brothers. The exceptional daughters were unevenly distributed over the 20 tested stocks. Six stocks produced no exceptional females at all; six produced these females at frequencies that were less than 1% of the total scored progeny, and the remaining eight produced them at higher frequencies. In one stock, the frequency of exceptional females was 11.08%.

A representative sample of 82 of the exceptional females were tested genetically to determine if they were cases of nondisjunction of the *X* chromosome, or if they had arisen through *X* chromosome attachment. All but one of these proved to be *bona fide* cases of *X* chromosome attachment. Six of the newly discovered attached-*X* chromosomes were examined cytologically using larval ganglion squashes. In all six, the *X* chromosomes were attached proximally to form reversed metacentric compound chromosomes. The structure of these compound chromosomes is shown in Figure 1. It is clear from the photographs that the compound chromosomes are the longest in the genome and that they result from the fusion of two apparently identical elements. At metaphase, each of these elements consists of two distinctly separated sister chromatids, plus a region in which no separation is evident. This latter region, comprising about 50% of the total length, is the proximal heterochromatin of the *X* chromosome. From the arrangement of this material on both sides of the centromere, it is clear that the compound chromosomes are reversed metacentrics. The classic double-*X* chromosome of MULLER (1943) is also shown in Figure 1 for comparison. This compound clearly does not have the reversed metacentric structure.

The results from this experiment demonstrate that among the stocks tested, exceptional females were produced at a high rate (1.81%, on average) and that almost all of these were due to *X* chromosome attachment. There was, however, considerable variation in the production of exceptional females among the stocks.

The results of an experiment to investigate this variation are given in Table 2. Males carrying *59b-z*, *Uc* and two different *Uc-l*^{r} chromosomes were tested for the production of attached-*X* females. The two *Uc-l*^{r} chromosomes used in this experiment had already been tested in the first experiment, and had produced exceptional females at a high rate.

The *59b-z* chromosome, from which the *Uc* and *Uc-l*^{r} chromosomes were derived, had a low ability to generate attached-*X* chromosomes; although only about 0.1% of the scored progeny were exceptional females, all of these turned out to be cases of *X* chromosome attachment. *Uc* was more potent at producing exceptional progeny; these amounted to about 0.6% of the flies that were scored, but because of the characteristic semisterility of homozygous *Uc*

TABLE 2

Formation of attached-*X* chromosomes in males carrying *59b-z*, *Uc* or *Uc-l^r* chromosomes

Chromosome	No. males tested	No. males with ≥1 exceptional daughter	No. sons	No. exceptional daughters	No. attached-*X*	No. not tested	Percent exceptional daughters ± SE
59b-z	122	9	9387	11	11	0	0.12 ± 0.04
Uc	64	15	3730	23	18	5	0.56 ± 0.16
Uc-l^r 10.5	61	18	2969	23	19	4	0.66 ± 0.15
Uc-l^r 16.41	77	50	4923	158	130	28	3.25 ± 0.49

Flies were counted until the 19th day after mating. The frequency of exceptional daughters is the unweighted average of the cultures scored.

females, five of these exceptions could not be tested further. Of the 18 that were tested, all proved to carry attached-*X* chromosomes, suggesting that most and possibly all of the exceptional females from this stock were cases of *X* chromosome attachment. One of the *Uc-l^r* chromosomes, *10.5*, produced exceptional females about as frequently as *Uc*, but the other, *16.41*, produced them at a much higher rate, about 3.2%. Of the 158 exceptions derived from this latter chromosome, 130 were tested for the attached-*X* condition, and all proved to have it. This suggests that the frequency of exceptional progeny from *Uc-l^r 16.41* is probably equal to the frequency of attached-*X* female production.

It is noteworthy that both of the *Uc-l^r* chromosomes produced exceptional females less often in the second experiment than in the first. The frequency of exceptional females dropped from 6.6% to 0.6% for *Uc-l^r 10.5* and from 6.9% to 3.1% for *Uc-l^r 16.41*. However, on account of the large standard errors in the first experiment, these changes are not statistically significant.

Recombinational analysis of *X* chromosome attachment: In an effort to determine if particular segments of these chromosomes were needed for attachment, *Uc-l^r* chromosomes were recombined with other *X* chromosomes not known to have a tendency to form compound chromosomes. Recombinant males were then studied for the production of attached-*X* females.

In the first recombination experiment, ten males from another *Uc-l^r* stock were mated to m/m females to produce *m*/*y^{59b} z w^i ct^6 f* heterozygous daughters. These were allowed to mate with their *m* brothers, yielding sons with various genotypes. We collected ten sons from each of seven genotypic classes from each of the ten matings, for a total of 700 males, each of which was tested for the production of attached-*X* females. These same males were also tested for the production of recessive *X*-linked lethal mutations, but the results of that work have been published elsewhere (LIM *et al.* 1983). Among the seven genotypes that we collected, two, *m* and *y^{59b} z w^i ct^6 f*, were parental and the other five were recombinant. These are listed in Table 3, which also gives the results of the experiment.

In each case, the frequency of attached-*X* chromosomes given is the frequency of exceptional females that proved to be cases of *X* attachment plus the fraction of exceptional females that could not be tested, but which, on the basis of those that were tested, could be assumed to carry attached-*X* chromosomes. The standard errors of these frequencies were determined by a method that incorporated an adjustment for untested flies [SIMMONS *et al.* (1980), p. 484]. Throughout the calculations, unweighted averages of the frequencies of individual cultures were used to obtain the estimates given in Table 3.

The frequency of attached-*X* females produced by males of the *Uc-l^r* base stock was determined just prior to the recombination experiment; the result was 0.73%, a value approximately equal to the frequencies for the *Uc* and *Uc-l^r 10.5* stocks reported above. Among the flies from the recombination experiment, the attached-*X* frequency for the *Uc-l^r* parental class (a in Table 3) was 0.38%, by a *t*-test not significantly less than the frequency for the base stock. The other parental class, *m* (d in Table 3), and four of the five recombinant classes (b, c, e and f) produced no attached-*X* females; however, the last of the recombinant classes (g) produced them at a low frequency (0.28%)

Qualitatively, these results suggest that at least two parts of the *Uc-l^r* chromosome are needed for attachment. This can be seen in Figure 2, which shows the structures of the recombinant chromosomes that were tested. Recombinant classes b and c both lacked a portion of the *Uc-l^r* chromosome proximal to *m*; in the case of c, the missing piece was greater than it was in b, but in both cases, at least the segment proximal to *f* was replaced by one from the *m* chromosome. Neither of these recombinant classes was able to form attached-*X* chromosomes, suggesting that something proximal to *m* (and probably proximal to *f*) was essential for *X* chromosome attachment. As mentioned above, cytological examination of newly formed attached-*X* chromosomes showed that attachment occurs at the proximal end. The apparent need

TABLE 3

Recombinational analysis of X chromosome attachment

Chromosome	No. males tested	No. males with ≥1 exceptional daughters	No. sons	No. exceptional daughters	No. attached-X	No. not tested	No. non-disjunctants	Percent attached-X ± SE
Experiment I:								
base (*y z w ct f*)	58	23	3791	28	23	4	1	0.73 ± 0.14
a (*y z w ct f*)	86	9	2886	12	9	3	0	0.38 ± 0.14
b (*y z w ct*)	85	0	2898	0	0	0	0	0
c (*y z w ct m*)	83	0	2746	0	0	0	0	0
d (*m*)	97	1	3953	1	0	0	1	0
e (*m f*)	100	2	4528	2	0	0	2	0
f (*f*)	98	2	3957	6	0	0	6	0
g (*ct f*)	84	9	3628	11	9	2	0	0.28 ± 0.10
Experiment II:								
base (*y z w ct f*)	471	51	7491	53	32	18	3	0.65 ± 0.10
Canton S	154	0	4055	0	0	0	0	0
1 (*ct f*)	394	32	10568	34	19	8	7	0.23 ± 0.05
2 (*cv ct f*)	477	11	8819	11	2	8	1	0.07 ± 0.02
3 (*cv f*)	333	4	8401	4	1	1	2	0.02 ± 0.01
4 (*cv v f*)	490	4	13162	4	2	0	2	0.02 ± 0.01
5 (*cv v*)	391	4	11508	4	0	3	1	0
6 (*cv v car*)	413	2	10612	2	0	1	1	0
7 (*y z w cv v car*)	437	0	10312	0	0	0	0	0
8 (*y z w v car*)	418	4	12656	4	2	0	2	0.02 ± 0.01
9 (*y z w ct v car*)	410	2	9958	2	0	1	1	0
10 (*y z w ct car*)	449	0	9580	0	0	0	0	0
11 (*y z w ct f car*)	364	1	7405	1	0	0	1	0
12 (*y z w ct f*)	463	37	9337	42	28	7	7	0.39 ± 0.08

Experiments I and II are described in the text. In each, flies were counted until the 16th day after mating. The frequency of attached-X females is the adjusted unweighted average, as described in the text.

for a proximal segment of *Uc-l^r* therefore may simply reflect a requirement for the attachment site itself.

Recombinant classes e and f both lacked a segment of *Uc-l^r* distal to *m*. In e the missing segment was greater than in f, but both lacked at least the piece distal to *ct*. Since neither could form attached-X chromosomes, some feature of *Uc-l^r* distal to *m* also appeared to be necessary for attachment.

In contrast to all the other recombinant classes, g formed attached-X chromosomes. The members of this class had the portion of *Uc-l^r* proximal to *ct*, but due to the uncertainty of the exchange point, might also have had a segment extending from *ct* to *w*. The fact that they did form attached-X chromosomes indicates that they had both the proximal attachment site and any distal elements needed for attachment.

In order to refine the analysis, we performed another recombination experiment in which additional markers were introduced to subdivide the *Uc-l^r* chromosome into smaller segments. *Uc-l^r* males were crossed en masse to females homozygous for *cv v car* to produce *cv v car/y^{59b} z w^i ct^6 f* daughters. These were then crossed to their *cv v car* brothers to produce an assortment of male progeny, from which 12 genotypic classes were selected for analysis. Four to six males of each class were crossed individually to *C(1)DX, y f/Y* females to establish lines. In some instances, these males were also mated to *cv v car* females to determine the complete genotype. This was necessary because *w^i* is epistatic to the other eye color markers. Two of the 12 classes, *y^{59b} z w^i ct^6 f* and *cv v car*, were the parental chromosome types; all the others were recombinants. Males from each chromosome line were tested for X chromosome attachment using established methods. The results are given in Table 3.

The rate of recovery of attached-X females from the *Uc-l^r* base stock was 0.65%; this estimate, obtained just prior to the recombination experiment, agrees well with the value obtained previously. For comparison, the Canton S stock was also tested for X chromosome attachment but no cases of attached-X chromosomes were found. In the recombination experiment, three of the 12 genotypic classes had nontrivial frequencies of attached-X females. One of these (12 in Table 3) was the *Uc-l^r* parental class, for which the frequency of attached-X females was 0.39%, nearly identical to that for the *Uc-l^r* class from the previous recombination experiment. Significantly, the other parental class (6) produced no proven attached-X females. The other two classes with nontrivial frequencies were 1 and 2, with rates of 0.23% and 0.07%, respectively. The latter value, however, reflects mainly the contribution of untested

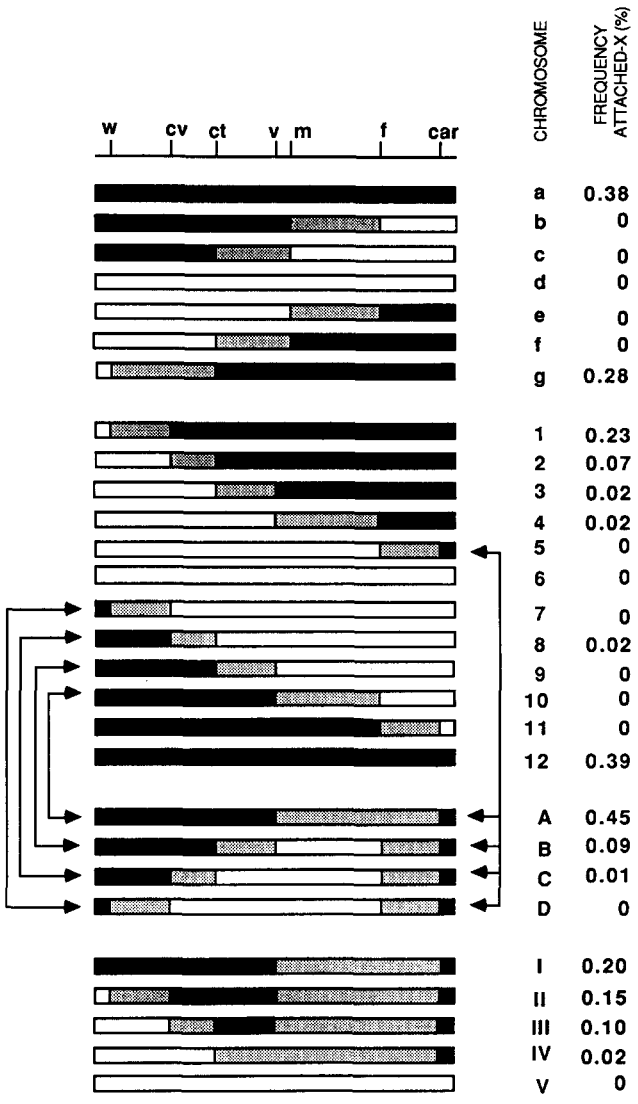

FIGURE 2.—Structures of the *X* chromosomes tested for the formation of compound chromosomes. The positions of the markers are shown at the top. The dark segments were derived from the *Uc-l*^{r} chromosome that was used in the recombination experiments. The light segments were derived from the *m* or *cv v car* chromosomes. The gray segments were of uncertain origin. The chromosomes in each group are identificed by letters or numbers. Chromosomes A–D were derived by recombination from some of the chromosomes shown above them in the figure; the *arrows* indicate how proximal and distal segments were recombined to generate A–D. The percentages of attached-*X* females obtained from each of the experiments are given in the column at the right.

exceptional females that were assumed to have carried attached-*X* chromosomes; this value may therefore be an overestimate. The other nine genotypic classes that were tested produced either very few (<0.02%) attached-*X* females, or none at all. The significance of these findings is best explained by considering the structures of the 12 classes of chromosomes, as shown in Figure 2.

Classes 7 through 11 all lacked proximal segments of *Uc-l*^{r}. Among them, only class 8 produced any attached-*X* females, but it did so at a very low rate (0.02%), suggesting that even this class lacked an element needed for *X* chromosome attachment. The chromosomes in class 11 were missing the smallest (and most proximal) piece of *Uc-l*^{r}, namely the segment to the right of *car*. In addition, these chromosomes lacked a segment of unknown length extending leftward from *car* to *f*. Because none of the males from class 11 produced any attached-*X* females, something on *Uc-l*^{r} proximal to *f*, and probably proximal to *car*, was necessary for *X* chromosome attachment. This finding reduced the uncertainty left from the first recombination experiment, which had put the essential proximal element somewhere to the right of *m*.

Classes 1 through 5 lacked distal segments of *Uc-l*^{r}. Class 1 males, which lacked only a tiny piece of *Uc-l*^{r} to the left of *w* plus a piece of unspecified length extending toward *cv* from *w*, produced attached-*X* females at a rate of 0.23%; clearly they had the ability to form attached-*X* chromosomes. Males from the other classes, however, appeared to lack this ability. The estimated frequency of attached-*X* females for these classes was ≤ 0.02%, except for class 2, where it was 0.07% (possibly an overestimate for the reason mentioned above). This suggests that if there is a unique distal element on the *Uc-l*^{r} chromosome that facilitates compound chromosome formation, it is located to the left of *ct*; otherwise, class 2 would have produced a higher frequency of attached-*X* females than it did. Alternatively, there might be several distal elements, each with a small effect, located between *w* and *v* on *Uc-l*^{r}. On this model, the class 2 chromosomes would have had only some of these elements, namely those located to the right of *ct*, thereby accounting for their limited ability to form compounds.

Reconstruction of *X* chromosomes capable of attaching from nonattaching *X* chromosomes: The recombination experiments suggested that a proximal segment of *Uc-l*^{r} located to the right of *f*, and probably to the right of *car*, was necessary for the formation of attached-*X* chromosomes. They also suggested that a distal segment, located somewhere to the left of *v*, was likewise needed. To demonstrate conclusively the need for these two segments, we attempted to produce a chromosome that could form compounds from two chromosomes that could not. The material for this project came from the experiment just described. A nonattaching chromosome from each of classes 7, 8, 9 and 10 was recombined with one from class 5. This last chromosome presumably carried the necessary proximal segment but lacked the distal one. The others might have had the distal segment, but definitely lacked the proximal one. By selecting recombinants between these two

TABLE 4

Reconstruction and recombinational analysis of X chromosomes capable of attaching

Chromosome	No. males tested	No. males with ≥1 exceptional daughters	No. sons	No. exceptional daughters	No. attached-X	No. not tested	No. non-disjunctants	Percent attached-X ± SE
Reconstruction experiment:								
A (*y z w ct*)	1053	89	22332	112	64	28	20	0.45 ± 0.07
B (*y z w ct v*)	1099	64	28071	73	19	12	42	0.09 ± 0.02
C (*y z w v*)	1106	3	35121	3	2	0	1	0.01 ± 0.01
D (*y z w cv v*)	1125	3	26564	4	0	2	2	0
Recombination experiment:								
I (*y z w ct*)	603	39	15005	46	28	6	12	0.20 ± 0.04
II (*ct*)	623	52	20456	61	25	11	25	0.15 ± 0.04
III (*cv ct*)	590	24	11905	24	4	15	5	0.10 ± 0.03
IV (*cv*)	600	19	22085	19	3	3	13	0.02 ± 0.01
V (*cv v car*)	593	4	19559	4	0	2	2	0

Flies were counted until the 16th day after mating. The frequency of attached-X females is the adjusted unweighted average, as described in the text.

types of chromosomes, we attempted to produce a chromosome that could form compounds.

The method was to cross males from one of the class 5 lines with *FM7/sc*^{7} *l* females to produce *FM7/5* balanced heterozygotes. These were then crossed with males from the other classes to produce heterozgotes between the class 5 chromosome and the other chromosomes. These heterozygotes were mated to their *FM7* brothers and recombinant sons were selected. From the *5/7* heterozygotes, we selected y^{59b} *z* w^{i} *cv v* sons, from the *5/8* heterozygotes, y^{59b} *z* w^{i} *v* sons, from the 5/9 heterozygotes, y^{59b} *z* w^{i} *ct v* sons, and from the *5/10* heterozygotes, y^{59b} *z* w^{i} *ct* sons. The selected males were mated individually to *C(1)DX, Y f/Y* females to establish lines and also to *cv v car* females to determine the correct genotype. For each recombinant class, 15 lines were established and then tested for X chromosome attachment.

Table 4 gives the results of the tests. Class A, from the recombination of chromosome *5* with chromosome *10*, produced many attached-X females; the estimated frequency was 0.45%. All 15 class A lines produced at least one *bona fide* attached-X female. As Figure 2 shows, the chromosomes in this class carried both a distal and a proximal segment from *Uc-l*^{r}, but the region between *v* and *car* was of uncertain origin. The performance of these class A recombinants therefore clearly demonstrates that the ability to form compounds can be restored by joining the left portion of chromosome *10* with the right portion of chromosome *5*.

The chromosomes of class B also were able to form compounds, although at a lower rate than the chromosomes of class A. These class B chromosomes came from the union of the left portion of chromosome *9* with the right portion of chromosome *5*. The segment between *m* and *f* was not derived from *Uc-l*^{r}, but the segments to the left of *ct* and to the right of *car* definitely were. As Figure 2 shows, the segments between *ct* and *m* and between *f* and *car* were of uncertain origin. If a unique distal element needed for X attachment were located to the left of *ct*, we would expect the class B recombinants to form compounds as frequently as the class A recombinants. Since this expectation was not met, it seems that some feature of *Uc-l*^{r} to the right of *ct* is needed for compound chromosome formation. This conflicts with the results of the second recombination experiment, in which the class 2 chromosomes suggested that an element was located to the left of *ct*. These latter chromosomes possessed the portion of the *Uc-l*^{r} chromosome to the right of *ct*, but formed very few compounds. The only way to reconcile these results is to propose that elements are present on both sides of *ct*, and that the low frequencies of attached-X females obtained with classes 2 and B are the result of an incomplete set of elements.

Class C produced only two attached-X females and class D did not produce any. Obviously, these chromosomes lacked the distal elements on *Uc-l*^{r} that are necessary for X chromosome attachment.

Recombinational analysis of a reconstructed attaching X chromosome: The results of an analysis of recombinants derived from one of the class A chromosomes are given in Table 4. These were obtained from *cv v car/*y^{59b} *z* w^{i} ct^{6} heterozygotes, which were themselves produced by crossing males from one of the 15 class A lines with *cv v car* females. Three recombinant and two parental classes were selected from among the male progeny of the heterozygotes. Each class was represented by 12 independent lines, which were maintained using *C(1)DX, y f/Y* females. Males from one class of lines (I) were crossed to *cv v car* to verify their genotype.

Class I, the parental type expected to produce attached-X females, did so, but at only half the rate

of class A, from which it was derived. Classes II and III also produced attached-*X* females, but at still lower rates than class I; nonetheless, the rates for these three classes were not significantly different from each other. Class IV produced only a few attached-*X* females, and class V did not produce any. These results suggest that some of the distal elements of *Uc-l*^{r} needed for *X* chromosome attachment were present in classes I, II and III, but not in classes IV and V. Figure 2 shows the structures of these chromosomes, which, except for class V, all had the proximal element that is required for attachment.

DISCUSSION

The *59b-z*, *Uc* and *Uc-l*^{r} *X* chromosomes form compound chromosomes at frequencies greater than 0.1%. In some experiments, the frequency was as high as 11%. Cytological examination indicated that these compound chromosomes were reversed metacentrics. Because these compounds were derived from males, each of them was evidently formed by recombination between sister chromatids, either in the male germline or in the zygote shortly after fertilization. However, the clustered appearance of these attached chromosomes among the offspring of individual males suggests a premeiotic origin. Furthermore, the high frequency with which these chromosomes were recovered in some experiments suggests that a recombinogenic factor was involved.

Genetic analysis of a *Uc-l*^{r} chromosome indicated that two segments were needed for compound chromosome formation. One of these was situated proximal to *forked* and probably proximal to *carnation* and very likely contained the attachment site. The other was located between *crossveinless* and *vermilion* and appeared to contain multiple factors influencing the attachment process. When either of these segments was removed by recombination, the *Uc-l*^{r} chromosome lost its ability to form compounds.

Uc and *Uc-l*^{r} chromosomes exhibit other unusual genetic properties. Both are highly mutable, as is clear from their tendency to acquire recessive lethal mutations at rates of 2–5% per generation (LIM 1979; LIM *et al.* 1983). They also tend to accumulate structural rearrangements, with breakage concentrated in polytene bands 6F1-2 (LIM 1979, 1981b). Many of these breakage events are also associated with recessive lethal mutations. The *Uc-l*^{r} chromosomes have been shown to impart high mutability to stable *X* chromosomes that have been paired with them for one generation (LIM *et al.* 1983). More recent studies have shown that these same *Uc-l*^{r} chromosomes can lose their high intrinsic mutability after many generations of laboratory culture (SIMMONS *et al.* 1985, 1986). They also lose the ability to destabilize other *X* chromosomes (SIMMONS *et al.* 1985).

The genetic instability of these chromosomes seems to be due to at least two factors. One is the retrovirus-like transposable element *gypsy* (MODOLELL, BENDER and MESELSON 1983), which JACK (1985) has shown to be associated with many of the *Uc*-induced recessive lethal mutations of the *cut* locus. All of these mutations occurred on chromosomes that had been destabilized by a *Uc* or a *Uc-l*^{r} chromosome (LIM *et al.* 1983; J. K. LIM, J. JACK and B. JUDD, unpublished data). It appears, therefore, that the process of homologue destabilization involves the mobilization of the *gypsy* transposon.

The other factor that is responsible for the instability of the *Uc* and *Uc-l*^{r} chromosomes acts principally in the 6F1-2 bands. Preliminary *in situ* hybridization experiments by one of us (J.K.L.) have established that the mutations and structural rearrangements at this site are associated with a genetic element sharing homology with the transposon known as *hobo*. This transposon comprises a heterogenous family, whose largest members seem to be about 3 kb in length (STRECK, MACGAFFEY and BECKENDORF 1986). Genetic and molecular analysis has implicated this transposon family in other genetic instabilities (BLACKMAN *et al.* 1987; YANNOPOULOS *et al.* 1987).

It is not clear what relationship exists between the mutational and breakage events on these chromosomes and their tendency to form compound chromosomes. The ability to form compounds, however, can persist in spite of a loss of mutational instability. This is shown by the data of SIMMONS *et al.* (1985), which involved the same *Uc-l*^{r} chromosome that was analyzed in the recombination experiments described here. SIMMONS *et al.* found no evidence for mutational instability, indicating that this chromosome had stabilized. Yet, as this paper reports, it has retained its ability to form compounds, although at a rate much less than LIM (1981a) had originally observed for other *Uc-l*^{r} chromosomes. A low rate of compound chromosome formation has consistently been a characteristic of this *Uc-l*^{r} chromosome, even when it was mutationally unstable (LIM *et al.* 1983).

It is nevertheless tempting to speculate that the formation of compound chromosomes might be linked to the instabilities of the *Uc* and *Uc-l*^{r} chromosomes because these instabilities are concentrated in the vicinity of the *cut* locus, which is located in one of the two regions needed for compound chromosome formation. It is possible that the transposons responsible for these instabilities exert an influence on chromosome behavior, perhaps by fostering a prolonged association between sister chromatids so that they can become attached. If these transposons also produce proteins that interact with DNA, it is conceivable that they might facilitate recombination in the proximal heterochromatin, where other transposon copies might be located, and thereby catalyze

the actual attachment events. All of these ideas must await a molecular understanding of the instabilities of the *Uc* and *Uc-l*^{r} chromosomes in order to be explored more fully.

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LITERATURE CITED

BLACKMAN, R. K., R. GRIMAILA, M. M. D. KOEHLER and W. M. GELBART, 1987 Mobilization of hobo elements residing within the decapentaplegic gene complex: suggestion of a new hybrid dysgenesis system in *Drosophila melanogaster*. Cell **49:** 497–505.

JACK, J. W., 1985 Molecular organization of the *cut* locus of *Drosophila melanogaster*. Cell **42:** 869–876.

LAVERTY, T. R. and J. K. LIM, 1982 Site-specific instability in *Drosophila melanogaster:* evidence for transposition of destabilizing element. Genetics **101:** 461–476.

LIM, J. K., 1979 Site-specific instability in *Drosophila melanogaster:* the origin of the mutation and cytogenetic evidence for site specificity. Genetics **93:** 681–701.

LIM, J. K., 1981a Patroclinous attached-X females in *Drosophila melanogaster*. Genetics **97:** s66.

LIM, J. K., 1981b Site-specific intrachromosomal rearrangements in *Drosophila melanogaster:* cytogenetic evidence for transposable elements. Cold Spring Harbor Symp. Quant. Biol. **45:** 553–560.

LIM, J. K., M. J. SIMMONS, J. D. RAYMOND, N. M. COX, R. F. DOLL and T. P. CULBERT, 1983 Homologue destabilization by a putative transposable element in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **80:** 6624–6627.

LINDSLEY, D., and E. GRELL, 1968 *Genetic Variations of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

LINDSLEY, D. L., and L. SANDLER, 1963 Construction of the compound-*X* chromosomes in *Drosophila melanogaster* by means of the Bar Stone duplication. pp. 390–403. In: *Methodology in Basic Genetics*, Edited by J. W. BURDETTE. Holden-Day, San Francisco.

MODOLELL, J., W. BENDER and M. MESELSON, 1983 *Drosophila melanogaster* mutations suppressible by the suppressor of Hairy-wing are insertions of a 7.3 kilobase mobile genetic element. Proc. Natl. Acad. Sci. USA **80:** 1678–1682.

MORGAN, L. V., 1922 Non-criss-cross inheritance in *Drosophila melanogaster*. Biol. Bull. **42:** 267–274.

MULLER, H. J., 1943 A stable double *X* chromosome. Drosophila Inform. Serv. **17:** 61–62.

NOVITSKI, E., 1963 The construction of new chromosomal types in *Drosophila melanogaster*. pp. 381–389. In: *Methodology in Basic Genetics*, Edited by J. W. BURDETTE. Holden-Day, San Francisco.

SIMMONS, M. J., N. A. JOHNSON, T. M. FAHEY, S. M. NELLETT and J. D. RAYMOND, 1980 High mutability in male hybrids of *Drosophila melanogaster*. Genetics **96:** 479–490.

SIMMONS, M. J., J. D. RAYMOND, M. J. BOEDIGHEIMER, E. A. DRIER, G. J. KOCUR, R. J. MORRISON and J. R. ZUNT, 1985 Stabilization of unstable *X* chromosomes. Drosophila Inform. Serv. **61:** 157–159.

SIMMONS, M. J., J. D. RAYMOND, E. A. DRIER and G. J. KOCUR, 1986 Further studies on unstable *X* chromosomes. Drosophila Inform. Serv. **63:** 125–126.

STRECK, R. D., J. E. MACGAFFEY and S. K. BECKENDORF, 1986 The structure of *hobo* transposable elements and their insertion sites. EMBO J. **5:** 3615–3623.

YANNOPOULOS, G., N. STAMATIS, M. MONASTIRIOTI, P. HATZOPOULOS and C. LOUIS, 1987 *hobo* is responsible for the induction of hybrid dysgenesis by strains of *Drosophila melanogaster* bearing the male recombination factor *23.5MRF*. Cell **49:** 487–495.

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