AN ABSTRACT OF THE THESIS OF

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(Name) (Degree) (Major)

Date thesis is presented August 19, 1963

Title INDUCTION OF GROSS DELETIONS IN RING-X AND ROD-X CHROMOSOMES OF DROSOPHILA MELANOGASTER (MEIGEN)

Redacted for Privacy

Abstract approved _____
(Major Professor)

An attempt was made to discover whether the theoretical difference in ring and rod chromosome reunion, following breakage, exists in Drosophila melanogaster (Meigen).

Males of a ring-X stock, X^{c2} ec f, and of a rod-X stock, Oregon R (wild type), were irradiated with 4500 r. in order to produce multiple chromosome breaks. These males were mated to $\overline{XX}$, Df(1)60g, y Hw · B f dl-49 v m w^a females. The resulting offspring should have been XO males, and y^+ females resulting from an X-chromosome to X-chromosome reunion forming a new duplication to cover the deficiency in the female.

The offspring of this experiment resulted in that expected, plus y females, nondisjunction females, and triploids. The occurrence of these unexpected events, due to irregular meiotic events, made it necessary to select the data for regular meiotic events. The

frequency of deletions for all series was .0020 for the rod and .0014 for the ring. However, the vast majority of the deletions were recovered in the last three irradiation series. Within these later series of irradiations the frequencies were .0051 for the rod and .0025 for the ring. This late series shows a trend which indicates less recovery of ring duplications than rod, though the data are too few to be substantiated statistically.

The results have value in that a frequency of induced deletions, not to be found in the literature, and a better understanding of the $\overline{XX}$, Df(1)60g genetics were obtained which will aid in the design of further experiments.

INDUCTION OF GROSS DELETIONS IN RING-X AND ROD-X CHROMOSOMES OF *DROSOPHILA MELANOGASTER* (MEIGEN)

By

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A THESIS

submitted to

OREGON STATE UNIVERSITY

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

August 1963

APPROVED: [redacted]

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Date thesis is presented August 19, 1962

Typed by Dora A. Maddox

ACKNOWLEDGEMENTS

I wish to express my thanks and appreciation to Dr. James D. Mohler of the Oregon State University zoology staff for his suggestion and assistance in this investigation.

The criticism and advice of Dr. Q. D. Clarkson and Mr. J. C. Neeley has been most helpful and to them I extend my thanks.

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INTRODUCTION

In Drosophila melanogaster, X-irradiation produces genetic mutations, some of which are associated with chromosome aberrations and some of which are not. The former are a product of chromosome breakage, while in the latter (point mutation) this has been questioned (13, p. 496-507).

It is known that if males bearing a ring-X chromosome are irradiated with X-rays and mated to normal females, there will be a deficiency of daughters among the offspring. The same is true for an irradiated rod-X chromosome male, but the depression of the sex-ratio is not as great (3, 4, p. 407-458, and 7, p. 25-40). Figure 1, depicts the events which can take place when a single break is induced in either a ring-X or a rod-X chromosome. Since Catcheside and Lea (7, p. 38-39) demonstrated that two-armed X-chromosomes are not recovered from irradiated sperm containing a ring-X, i.e., one due to healing of telomeres, the broken ends must rejoin and this may be accomplished by way of the events shown in Figure 1. Two of these ways lead to loss of the ring-X chromosome; which is not true for a rod-X chromosome, as only one of the three events leads to loss. The interpretation of the greater sex-ratio depression in the case of the ring-X versus rod-X has been explained in such a manner.

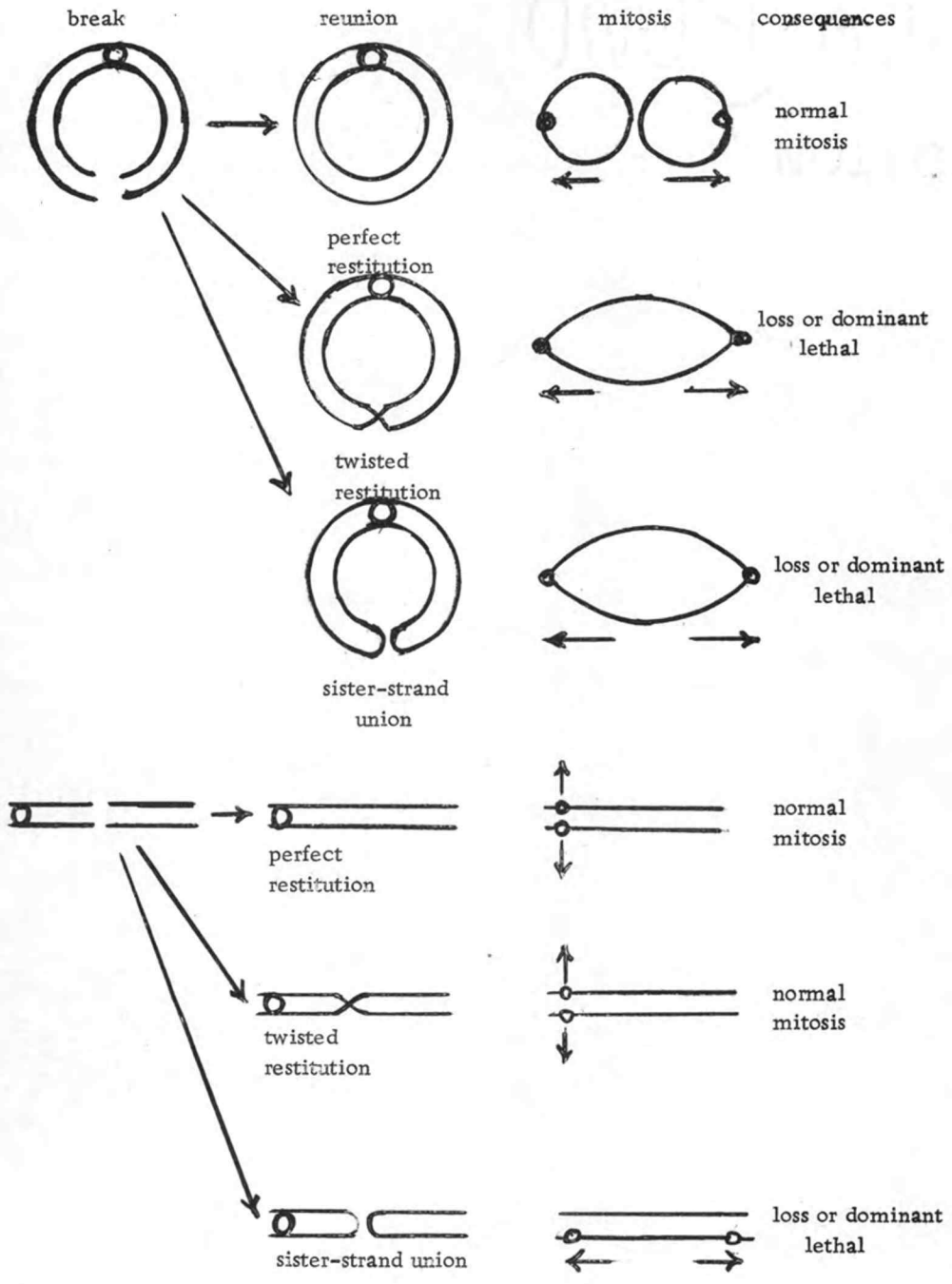

Figure 1. Hypothesized reunion events following breakage in ring-X and rod-X chromosomes.

It would be expected that if mutation were due to breakage of the X-chromosome, there would be a lower frequency of recovered mutations in a ring-X than in a rod-X. Studies of Novitski (16) and of Offerman and Muller (11, p. 40-41) show no decrease in frequency of sex-linked lethal mutations. The same sort of result for rings and rods has also been found in the case of visible mutations not associated with detectable chromosome changes (12, p. 27-30). These results have been accepted as showing that the great majority of mutations arise independently of breakage and restitution (13, p. 498).

However, it has been shown (12, p. 27-30) that the frequency of small deletions is also not lower in recovered irradiated rings when compared with rods. Since small deletions are most reasonably interpreted as the result of at least a single chromosome break, other questions immediately come to mind: perhaps reunited rings are not lost more frequently than reunited rods; or perhaps there are compensating mechanisms in the ring-X chromosome which account for the similarity of the data cited.

With this in mind the present study was conducted. Gross deletions, which are unequivocably due to chromosome breaks, were induced in both ring-X and rod-X in order to determine whether there is a greater loss of ring duplications than rod duplications.

METHODS AND MATERIALS

Male Drosophila melanogaster were placed in gelatin capsules and irradiated in air. The irradiation was produced by a Standard Mobile Oil-cooled Therapy Unit with a Eureka TG3 Oil-immersion Therapy Tube, with a target of tungsten imbedded in cooper. The dosage of 4500 r. was obtained by using a tube voltage of 8 MA with an input power of 125 KVP, which without a filter and twenty centimeters from the capsules, produces approximately 95 r. per minute. The dosage was checked with a Victoreen model 70 roentgen meter.

Immediately after irradiation each male was mated to five virgin females. Matings were carried out in 25 by 95 mm shell vials containing the standard corn meal, molasses, agar medium and incubated at 25° C. The parent flies were transferred to new vials every two days.

The males used in this study were Xc^2 ec f (a ring-X chromosome stock) and Oregon R-180 (a wild type stock containing a rod-X chromosome; see Table 1 for a list of mutant alleles discussed in this paper). The females used contained a reversed-acrocentric X-chromosome of the constitution $\overline{XX}$, Df(1)60g, y Hw · B f dl-49 v m w^a sc^8.

This attached-X contains a deficiency which includes most of the proximal heterochromatin and the su-f locus, and has the ac to y

Table 1. A listing of the mutant alleles and the phenotypic character affected, referred to in this study (6, p. 239-240).

Genetic Symbol	Name of Mutant	Character Affected
ac	achaete	bristle number
y, y^{31d}	yellow	body color
Hw	Hairy-wing	bristle, hair number
sc	scute	bristle number
w, w^{a}	white, apricot	eye color
ec	echinus	facet size
cv	crossveinless	wing veination
v	vermilion	eye color
m	miniature	wing size
f	forked	bristle shape
B	Bar	eye shape
su-f	suppressor of forked	bristle shape

(mutants are listed in order of their respective loci on the standard map).

region represented but once (10). Diploid females containing this reversed-acrocentric attached-X chromosome do not survive unless the deficiency is covered. The stock is maintained with the duplication carried by males which have the genotype $\overline{XY}$, y v/Dp(1)60g y^{31d}, so that viable diploid females of this stock have the phenotype y^{31d} Hw · B.

When males of either the ring-X or rod-X stocks are mated to the attached-X females from the stock, described above, the surviving offspring should consist only of XO males, phenotypically like their male parent, and metafemales (see Table 2). Since meta or superfemales are rarely viable the XO males are the major expectation.

A classical system for scoring gross deletions, induced by X-ray treatment in the males, is to measure the frequency of y^+ daughters of the treated y^+ males mated to attached-X y females. The The attached-X used in this study has the advantage that the recovered fragments in the y^+ females should arise in the following manner: a "two hit" break in the X-chromosome of either type of male may cause the production of a fragment which will cover the deficient region in the attached-X chromosome. In this way recovery of reunion only of X-chromosome to X-chromosome is assured. These y^+ fragments would be deficient for a large part of the X-chromosome, but would contain that portion missing in the attached-X chromosome (see Figure 2).

Table 2. Types of progeny produced when a ring-X or rod-X male is mated to females containing the reversed-acrocentric attached-X chromosome.

Male gametes (ring or rod)	Female Gametes: $\overline{XX}$, Df(1)60g	Female Gametes: Dp(1)60g
X	$X/\overline{XX}$, Df(1)60g metafemale	X/Dp(1)60g XO male, survive but sterile
Y	$\overline{XX}$, Df(1)60g/Y dies, deficient segment not covered	Dp(1)60g/Y dies, no X-chromosome

Unirradiated ring-X and rod-X males mated to the attached-X females were used as controls.

To establish stocks of the duplications each exceptional female was mated to the $\overline{XY}$ males containing the duplication, so that the males from this cross would contain $\overline{XY}$/ y^+ fragment. These $\overline{XY}$/ y^+ fragment males were mated back to the $\overline{XX}$, Df(1)60g stock to place the fragment in the females, who were then mated back to the $\overline{XY}$/ y^+ fragment parent and a stock containing the y^+ fragment obtained. It is now possible with these stocks to make appropriate crosses to determine the breakage points, however, these progeny tests have not been completed so that the actual loci included in the deletions are still not known.

During the course of the experiment egg counts were made in order to determine the percentage of eggs hatching in both the ring-X and rod-X. Matings, both irradiated and unirradiated, as described above, were carried out and the number of eggs produced counted, with a subsequent count of the number which produced pupae. From Table 2 it would be expected that 1/4 of the eggs should hatch.

Statistical analysis of the data was carried out according to the the methods outlined in Li (9, p. 390-431) for binomial populations; the tests used were the "u" and X^2 .

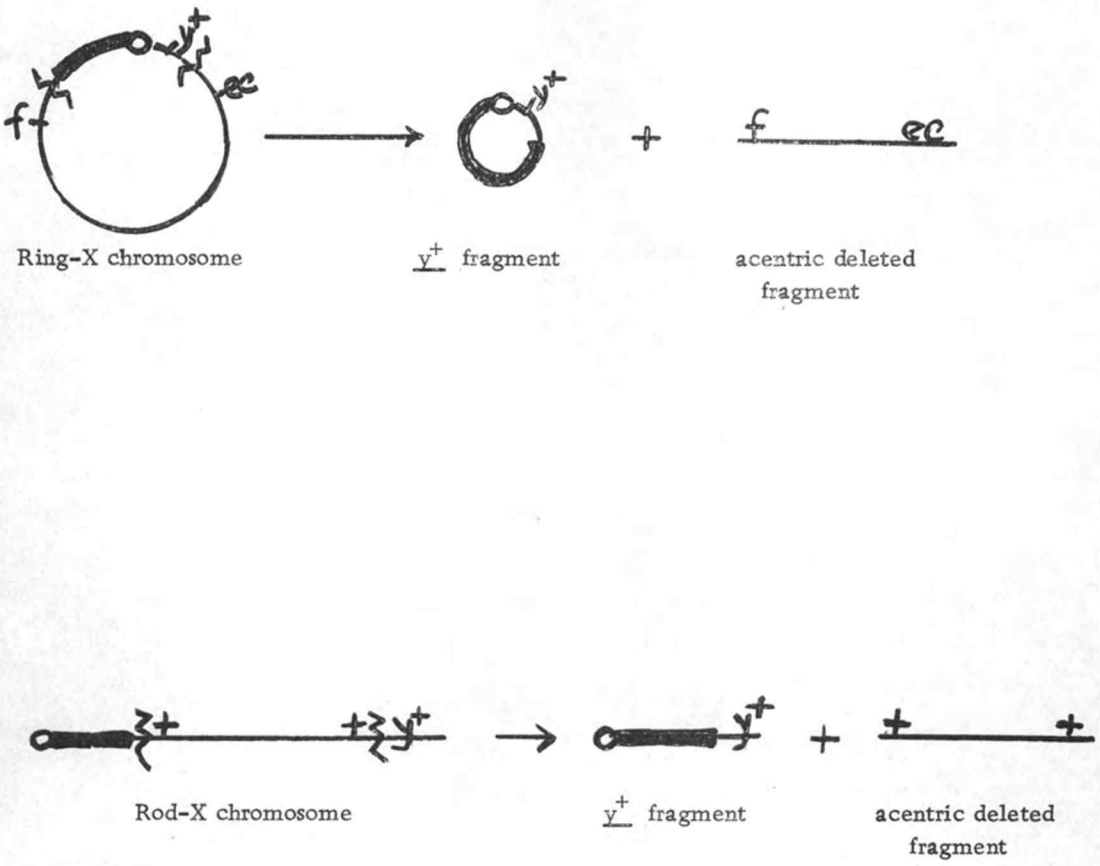

Figure 2. Manner of production of gross deletions and yellow-plus fragments from ring-X and rod-X chromosomes by fragmentation with X-rays.

RESULTS

The F_1 progeny of each of the matings were classified as to phenotype and possible event required for their origin (see Table 3). The results indicated that several events not expected, such as the occurance of a high frequency of nondisjunction females and the surprisingly high frequency of triploids and intersexes, were occuring in addition to those exceptional events predicted.

UNPREDICTED EVENTS

Females which were $\underline{y^{31d}}$ $\underline{Hw}$ · $\underline{B}$ in phenotype, like their mothers, indicated that nondisjunction was taking place. Such females arise as the failure of the attached-X and the duplication to separate at meiosis. When eggs of this type are fertilized by a Y-chromosome bearing sperm they give rise to matroclinous females.

Females of the Phenotype $\underline{y^{+}}$ $\underline{Hw}$ · $\underline{B}$, which was the phenotype expected of the deletion events, were also observed; these, however, were not due to deletion events but were recognized as triploids due to a slight difference in phenotype from the diploid, and by progeny testing. Intersexes which are the complementary class to the triploids, were recognized by their phenotype which was $\underline{y^{31d}}$ $\underline{Hw}$ · $\underline{B}$, like their mothers, but the sex-phenotype was male-like. Triploids and intersexes both arise from eggs which are unreduced,

Table 3. Numbers of flies of the various types, based on the data found in the appendix.

	Rod-X		Ring-X	
	Control	X-ray treated	Control	X-ray treated
	No. of individuals	No. of individuals	No. of individuals	No. of individuals
XO	997	2542	1109	2909
Deletions				
y^+	0	5	0	4
y	0	3	0	4
Nondisjunction females	10	29	18	45
Metafemales	3	1	0	2
3N females	2	0	4	2
Intersex	0	0	2	0
Total Number of individuals	1012	2580	1133	2966

i. e., the entire diploid set of chromosomes is present in the egg. They differ in that triploids arise from the fertilization of such eggs by a X-chromosome bearing sperm, and the intersexes by fertilization with a Y-chromosome bearing sperm.

Since the inclusion of these unpredicted exceptional events distorts the ratio of deletions to XO males they are excluded when determining the frequency of deletions. Some families, shown in the detailed data of the appendix, are not included in Table 3, as the frequency of the exceptional events was extremely high. The reliability of the data is made less secure if a nondisjunction female or a triploid female, either of which may have arisen in the $\overline{XX}$, Df(1)60g stock, was used as a maternal parent. Such females, if one of the mothers, would yield a higher frequency of exceptional events, discussed above, as compared with regular cultures.

The consequences of nondisjunction and the occurance of unreduced eggs is to increase the apparent frequency of deletions. A study of Table 4 reveals that distal fragments of the X-chromosome fertilizing a nondisjunction egg will show up as an increase in deletion frequency. Similarly fertilization of unreduced eggs by such fragments will lead to intersexes of the desired deletion phenotype which if female-like might be included in the frequency of deletions. This latter event can be ignored, however, since the occurance of

Table 4. Possible mode of origin of the exceptional events.

Type of Male Gamete	Normal Eggs Dp	Normal Eggs $\overline{XX}$	Nondisjunction Eggs ---	Nondisjunction Eggs $\overline{XX}/Dp$	Unreduced Eggs 2N	Unreduced Eggs ---
X	XO male	--	XO male	--	3N female	--
* X to X fragments						
y^+	--	y^+ female	--	y^+ female	y^+ intersex	--
y	--	y female	--	y^{31d} female	y^{31d} intersex	--
**X to A fragments						
distal-X	--	--	--	y^+ female	y^+ intersex	--
proximal-X	--	y female	--	y^{31d} female	y^{31d} intersex	--
Y	--	--	--	y^{31d} female	y^{31d} intersex	--

*Events desired for measurement.

**Half-translocations.

fragments of the X-chromosome is very low and the occurance of unreduced eggs is even lower. The effect of the former is evaluated in the discussion.

Among the progeny recognized as deletions not all were $\underline{y^+}$, as outlined in the methods, some were $\underline{y}$. These $\underline{y}$ deletions could have arisen in the same manner as the $\underline{y^+}$, the exception being that the break would necessarily have to be to the left of the $\underline{y}$ locus on the X-chromosome of the male. On the other hand, the $\underline{y}$ females could have arisen from the reunion of a proximal X-fragment with an autosome (A), yielding what are known as half-translocations (see Table 4; 1). The frequency of such events should be higher than the X to X type of reunion, as it is known that most breaks occur in the autosomes as compared with the X (8, p. 164). Since these cannot be determined to be due to deletion events without progeny testing, and since the progeny tests as of yet are not completed, these $\underline{y}$ females were not included when determining the frequencies of deletions.

DELETION FREQUENCIES

The frequency of presumed deletions ($\underline{y^+}$) in ring-X (. 0014) and in rod-X (. 0020), appear to be approximately equal, and the difference is not significant (u = . 4651). Heterogeneity in the data was was obvious when the frequency of all types of deletions ($\underline{y^+}$ and $\underline{y}$)

Table 5. Heterogeneous effect of X-ray treatment between the early and late irradiation series.

		Rod-X				Ring-X			
	Treatment	Series Number	XO Males	Deletions y^+	Deletions y	Series Number	XO Males	Deletions y^+	Deletions y
Early Irradiation	1	I	113	0	0	I	38	0	0
	2	-	-	-	-	II	225	1	0
	3	-	-	-	-	III	639	0	1
	4	II	1195	1	0	IV	568	0	0
	5	III	456	0	0	V	269	0	0
	Sub total		1764	1	0		1739	1	1
Late Irradiation	6	IV	778	4	3	VI	600	2	1
	7	-	-	-	-	VII	570	1	2
	Sub total		778	4	3		1170	3	3
	Grand total		2542	5	3		2909	4	4

in the early series was compared to that in the latter series (see Table 5). The frequency of presumed deletions ($\underline{y^+}$) in the ring was . 0025 and the frequency in the rod was . 0051 for the late series of irradiations, which is higher than for the early series of irradiations. The frequencies obtained in the late series do not differ significantly from a 2 to 1 ratio, i. e., that there is twice as many recovered deletions in the rod as in the ring (u = . 0026), however, the possibility that the frequencies are equal is not excluded by data of this magnitude (u = . 6760).

Since some of the early series of irradiations did not produce deletions, and the majority of all types of deletions ($\underline{y^+}$ and $\underline{y}$) arose in the late series, 13 out of 16, it would appear as if some of the early series were essentially untreated. The egg counts were used as an independent test of the X-ray effect, since one would expect that 1/4 of the eggs should hatch if there is 100 per cent viability of the XO males, a decrease in this value could be interpreted as due to dominant lethals. Since dominant lethals cannot be studied in successive generations we can only infer their existence in irradiated sperm, by observing that a certain frequency of eggs which are fertilized by dominant lethal bearing sperm fail to develop into mature flies. It is supposed by Bonnier and Luning (5, p. 445-456) that dominant lethals are induced by chromosome breaks, although a

Table 6. Frequency of eggs hatching from ring-X and rod-X sperm from both control and treated males

	Control			Treated		
	No. of eggs	Number hatched	%	No. of eggs	Number hatched	%
Rod-X	119	37	31.1	150	14	9.3
Ring-X	138	41	29.9	154	9	5.8

certain number of them may be point mutations, as it is impossible to discriminate between these two events.

The results of the egg count tests, shown in Table 6, clearly show that both types of males do have approximately 100 percent viability, and that they are being treated by X-rays, as the loss of rings is nearly twice as great as in rods (X^2 = .3498, 1d.f.), however, it does not exclude the possibility that the loss of rods is equal to rings (X^2 = 1.0956, 1 d.f). These data on dominant lethals in ring and rod, though not conclusive, support the results of Baker (2, p. 735-748) and of Muller and Pontecorvo (14) where it was shown that there is approximately twice as many rings lost as rods due to dominant lethals. From the data presented one can say that there is a trend in the later series of irradiations which is in the direction of a greater loss of rings than of rods.

DISCUSSION

The trend in the late series, which suggested that fewer ring duplications than rod duplications were recovered, is in agreement with the results expected upon the basis of the predicted reunion events. If this trend can be substantiated, it would aid in the interpretation of experiments on recessive lethal mutations and small deletions. One could postulate that these events are not due to chromosome breakage. This is not conceptually difficult for gene mutations but still poses a problem when the small deletions are involved. It may be that there is some other mechanism acting in the recovery of small deletions which favors equality of loss, such as some difference in action after irradiation, or a compensating mechanism which, although breakage occurs, selectively favors the equality that has been reported (12, p. 27-30).

It is possible that the small differences, observed in these experiments, could be explained by mechanisms other than those proposed by the predicted reunion events. The original design of this experiment, which did not anticipate the occurance of nondisjunction, assumed that half-translocations which produce non viable $\underline{y}^{+}$ females would not add to the frequency of presumptive deletions. The occurance of $\underline{y}^{+}$ females which arise by the fertilization of a nondisjunction egg with sperm carrying a distal X-fragment reunited with an autosome (half-translocation), as shown

in Table 4, will bias the data in favor of greater ring loss. This is true because events of this type would more easily take place in the rod than in the ring, since it would require more breaks to obtain such events in the ring than in the rod chromosome. Further, most half-translocations in the ring would lead to a loss of the ring chromosome. Since the occurance of nondisjunction and half-translocations is rare the amount of progeny testing necessary to identify them would be less using the present design than other possible designs.

One other difference in the ring and rod chromosomes should be mentioned. The size of the ring-X is somewhat larger than the rod-X (17). Consequently, one would expect that more breaks could occur in the ring than in the rod, which would lead to a slight difference in ring versus rod loss. A means of overcoming this would be to use the open ring described by Novitski as In(1)EN (15), which would be a rod chromosome of comparable size to the ring used in this study.

The present data also leave open the possibility that the observed differences may be due to chance. However, the deletion frequencies obtained in this study allow one to estimate the sample size needed to design an experiment to test whether there exists a real difference in ring duplication recovery as compared to rod

duplication recovery. Considering the frequencies of $\underline{y}^{+}$ alone, the sample size needed for all series taken together, which show a small difference in effects, would be approximately 46,000. On the other hand, the late series of irradiations, which suggest the large difference predicted, would be approximately 5,000 (these values are derived by solving for sample size in the "u" test, 9, p. 408).

Inconclusive as the results are, several things which will aid in the further investigation of this problem have been found. These include a frequency of deletion events, not to be found in the literature, which can be used as a criteria for the sample size design; and a further description of the $\overline{\underline{XX}}$, $\underline{Df(1)60g}$ stock, which will make it easier to predict certain phenomena which were observed to be taking place during the course of the experiment.

SUMMARY

(1) A comparative study was made of the recovery of duplications from fing, $\underline{X^{c2}}$ $\underline{ec}$ $\underline{f}$, and from rod, Oregon-R (wild type), chromosomes treated by irradiation of Drosophila melanogaster sperm.

(2) Irregular meiotic events were discovered in the egg stock ($\overline{\underline{XX}}$, Df(1)60g) used in this study. These included a relatively high frequency of nondisjunction and unreduced eggs, so that the data required selection for the regular meiotic events.

(3) The data from regular meiotic events show that there is a trend in the late series of irradiations which indicates less recovery of duplications in the ring than in the rod. However, the data are too few to give statistical significance to this trend.

(4) Half-translocations, which yield $\underline{y^+}$ females, may slightly bias the data in favor of greater rod duplication recovery than ring duplication recovery.

(5) The size of the rod-X, which is slightly smaller than the ring-X, may also account for a slight bias in favor of greater rod duplication recovery than ring duplication recovery.

(6) A sample size to discriminate between the frequencies obtained would require 46, 000 flies,for the small differences obtained in all series of irradiations; and 5, 000 flies to distinguish

between the larger difference, which was predicted, in the late series of irradiations.

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APPENDIX

Rod-X Control

Culture Number	No. of XO males per culuture	Nondisjunction females	3N females	Intersex	Meta-females
1	205	3			1
2	157				
3	108				
*4	167	2	10	10	
5	65	1			
6	104	1			
7	177	2	2		2
8	181	3			
Total	1164	12	12	10	3

Ring-X Control

Culture Number	No. of XO males per culture	Nondisjunction females	3N females	Intersex	Meta-females
1	63				
2	124	1			
3	143	1			
4	162	2	3		
5	180	3	1	2	
6	164	5			
*7	165	36			
8	104	4			
9	169	2			
Total	1274	54	4	2	0

* Culture numbers marked with an asterisk are not reported in the totals in table 3.

ROD-X TREATED

Culture Number	Number of XO Males Per Culture	Presumptive Deletions	Nondisjunction Females
	Series I		
1	4		
2	2		
3	3		
4	3		
5	0		
6	6		
7	8		1
8	14		
9	13		
10	2		
11	4		
12	5		
13	4		
14	29		
15	16		
Total	113	0	1
	Series II		
1	1		
2	0		
3	9		
4	47		1
5	45		
6	67		
7	3		
8	198		3
9	187		
10	0		
11	2		
13	68		
14	126		2
15	8		
16	62		2
17	148	1 ($\underline{Y}^{+}$)	
18	50		
19	110		
20	63		
Total	1195	1 ($\underline{Y}^{+}$)	8

ROD-X TREATED (continued)

Culture Number	Number of XO Males Per Culture	Presumptive Deletions	Nondisjunction Females
		Series III	
1	8		
2	24		
3	40		
4	24		
5	3		1
6	4		
7	4		
8	35		1
9	20		
10	33		
11	25		
12	27		
13	24		
14	31		3
15	22		
16	13		
17	5		
18	35		
19	25		
20	15		
21	39		1
Total	456	0	6

Also 1 metafemale in culture number 8.

		Series IV	
1	44		
2	0		
3	47	1 ($\underline{y}$)	
4	27		
5	50		
6	63		3
7	0		
8	79	1 ($\underline{y^+}$)	1
* 9	77		9
10	57	1 ($\underline{y^+}$)	
11	54		1
12	33		2
13	54		3
14	52		
15	55		
16	51	1 ($\underline{y^+}$)	
17	45	1 ($\underline{y}$)	2
18	0		
19	30		
20	37	1 ($\underline{y}$) 1 ($\underline{y^+}$)	2
Total	885	7 (3 $\underline{y}$ and 4 $\underline{y^+}$)	23

RING-X TREATED

Culture Number	Number of XO Males Per Culture	Presumptive Deletions	Nondisjunction Females
	Series I		
1	1		
2	2		
3	3		
4	2		
5	7		
6	2		
7	2		
8	3		
9	7		
10	4		
11	4		
12	0		
13	1		
14	0		
15	0		
Total	38	0	0

Also 1 metafemale in culture number 3.

	Series II		
1	21		
2	20		1
3	49		1
4	30		
5	31		
6	14		
7	29		2
8	31	1 (y^+)	1
9	0		
10	0		
Total	225	1 (y^+)	5

Also 1 metafemale in culture number 4.

RING-X TREATED (continued)

Culture Number	Number of XO Males Per Culture	Presumptive Deletions	Nondisjunction Females
		Series III	
1	117	1 (y)	3
2	10		
3	47		1
4	4		
5	10		1
6	15		
7	81		
8	149		
9	9		
10	13		1
11	43		1
12	48		2
13	93		
14	0		
15	0		
Total	639	1 (y)	9
		Series IV	
1	2		
2	125		1
3	3		
4	31		1
5	92		1
6	127		
7	25		
8	20		
9	126		2
10	17		
Total	568	0	5

RING-X TREATED (continued)

Culture Number	Number of XO Males Per Culture	Presumptive Deletions	Nondisjunction Females
1	3		
2	37		
3	26		
4	17		
5	23		
6	4		
7	15		
8	4		
9	1		
10	12		
11	5		
12	8		
13	3		
14	12		1
15	0		
16	23		
17	2		
18	28		1
19	24		
20	22		1
Total	269	0	3

RING-X TREATED (continued)

Culture Number	Number of XO Males Per Culture	Presumptive Deletions	Nondisjunction Females
		Series VI	
1	25		
2	18		
3	15	1 ($\underline{y}^+$)	1
4	58		1
5	7		1
6	36		1
7	9	1 ($\underline{y}$)	
8	16	1 ($\underline{y}^+$)	
9	12		1
10	25		
11	29		
12	31		1
13	26		
14	24		
15	2		
16	22		
17	1		
18	4		
19	11		
20	10		
21	27		
22	15		
23	8		
24	28		
25	25		2
26	7		
27	26		
28	11		
29	24		2
30	10		
31	22		1
32	16		
Total	600	3 (1 $\underline{y}$ and 2 $\underline{y}^+$)	11

Also two 3N females 1 each in culture numbers 1 and 25.

RING-X TREATED (continued)

Culture Number	Number of XO Males Per Culture	Presumptive Deletions	Nondisjunction Females
		Series VII	
1	17		1
2	18		
3	5		1
4	13		
5	7		
6	6		
7	14		
8	3		
9	14		
10	16		
11	15		1
12	27	1 ($\underline{y}$)	1
13	14		1
14	6		
15	5		
16	26		
17	32		1
18	21	1 ($\underline{y^+}$)	
19	12		
20	11		
21	9		
22	11		
23	12		
24	18		
25	14		
26	29	1 ($\underline{y}$)	
27	7		
28	13		1
29	10		
30	6		
*31	12		6
32	8		2
33	11		1
34	13		
35	18		
36	5		
37	12		
38	24		
39	25		1
40	9		
41	5		
42	15		1
43	14		
Total	582	3 (1 $\underline{y^+}$ and 2 $\underline{y}$)	19