A STUDY ON THE GENETIC EFFECTS OF IRRADIATING DROSOPHILA MELANOGASTER MALES WHICH CARRY RING-X CHROMOSOMES.

by

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INTRODUCTION

1) The preliminary experiments:

The problem which formed the starting point of the present investigation arose out of a group of preliminary experiments which were carried out in close co-operation with Professor Sobels (1963). In these experiments one-day old X^{C2},y B/ sc^{8}.Y Drosophila melanogaster males were irradiated in either oxygen or nitrogen and then immediately given a post-treatment in either of these two gasses. In oxygen a dose of 1kR was given and in nitrogen a dose of 3kR was given. To measure the effects of the different treatment combinations on the various germ cell stages, a brood pattern analysis was employed, with two-day broods and six females per males per brood. Sex chromosome losses, XO males, and recessive sex-linked lethals were the two classes of damage which were used for measuring the effectiveness of each radiation-gas combination. The results of these experiments are summarised in figures 1 and 2.

Following irradiation in oxygen (fig.1), neither the frequencies of XO males nor the frequencies of sex-linked lethals were differentially affected by post-treatment in oxygen or nitrogen. The highest frequencies of sex-linked lethals were recovered in brood C and the highest frequencies of XO males in brood D.

Exposure of the males to nitrogen, after 3kR given in nitrogen, (fig.2), produced a pattern of damage which was similar to the ones obtained in the oxygen-irradiation experiments. However, when irradiation in nitrogen was followed by an oxygen post-treatment there was a marked change in the brood pattern recovery of sex-linked lethals. There was no peak in brood C; the

frequency remained at one level through the first four broods and then decreased in the fifth brood. In this experiment the XO male peak was not eliminated, but it was slightly lower than the peak in the nitrogen post-treatment experiment ($0.05 > P$).

From these experiments it was concluded that -

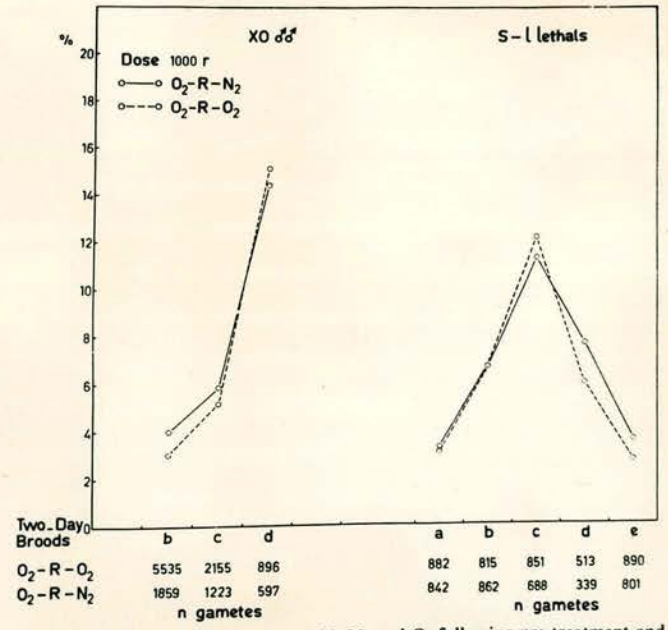
a) the germ cell stage which is the most sensitive to the induction of sex-linked lethals is older than the germ cell stage which is the most sensitive to the induction of XO males; the sex-linked lethal peak occurred in brood C and the XO male peak occurred in brood D.

b) following irradiation in nitrogen, the amount of genetic damage recovered is dependent on the gas used as an immediate post-treatment and the extent of relative modification is dependent upon the character used to measure radiation damage.

c) the oxygen effect affects the induction of both XO males and sex-linked lethals; that is, 3kR in nitrogen is equivalent to 1kR in oxygen as measured by these two types of genetic damage.

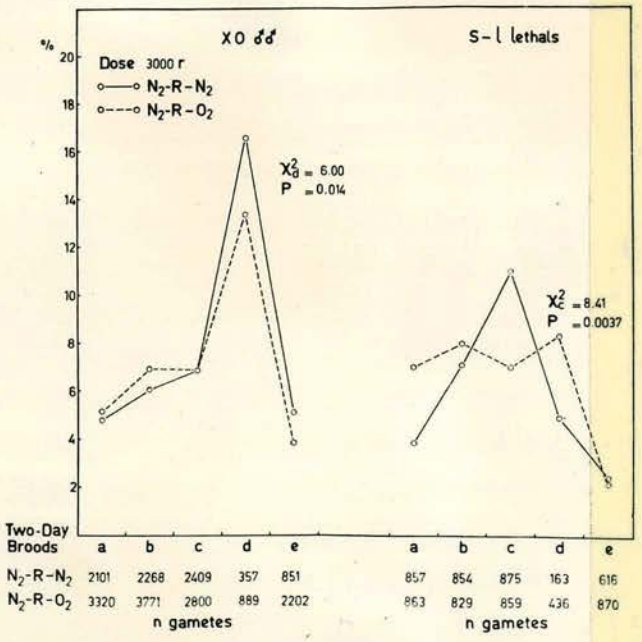
The observation that different broods contained the highest frequencies of sex-linked lethals and XO males was in agreement with the data which had been obtained after the irradiation of rod-X males (Savhagen 1961, Strangio 1962). Both of these authors concluded that chromosome breakage and loss was the main cause of XO males and that the chromosomes in spermatocytes were thus the most sensitive to breakage by X-rays. However, it was also possible that the high frequencies of XO males were caused by induced non-disjunction and crossing-over, two types of damage which cannot be induced in the post-meiotic spermatids which are the most sensitive stage for the induction of sex-linked lethals and translocations (see recent review by Mandl, 1964, section III(4)).

Figure 1. (taken from Sobels,1963).



The effect of post-treatment with N_2 and O_2 following pre-treatment and irradiation (1000 r) in O_2. Note that both post-treatments had the same effect. Frequencies of XO males are based on the total number of males. (Data of Sobels and Leigh).

Figure 2. (taken from Sobels, 1963).



The effect of post-treatment with N_2 and O_2 following pre-treatment and irradiation (3000 r) in purified N_2. The results for XO males (yellow exceptions) are shown left, those for recessive lethals, induced in a XC^2y B chromosome, right. Note the lowering effect of O_2-post-treatment for XO males in brood d and for lethals in brood c. Frequencies of XO males are based on the total number of males. (Data of Sobels and Leigh).

2) The problem:

The original problem was to determine whether chromosome breakage was the main cause of the high frequencies of XO males which occurred in the spermatocyte brood. The ring-X chromosome seemed to be an ideal tool for such an investigation because it was lost at a relatively much higher rate than rod-X chromosomes.

From the start, data were obtained which could not be satisfactorily explained by the chromosome breakage theory of chromosome loss and dominant lethality. Therefore further research was directed towards an investigation of the consequences of irradiating ring-X males. A standard treatment was compared with variations of this treatment, such as post-treatment with oxygen or using a different radiation dose rate, and the genetic effects were measured by the frequencies of XO males and the differential pre-adult lethality of male and female zygotes.

3) The theory of sex ratio shift:

At the start of the present investigation, the theory of radiation induced chromosome loss formed part of the general theory of dominant lethality and sex ratio shift (Muller 1940; Pontecorvo and Muller 1941: Pontecorvo 1940, 1941 and 1942). The dose-effect curves for the induction of dominant lethals, in mature sperm, followed one-hit kinetics at low doses and at higher doses there was evidence of an increasing multihit component. This was illustrated by Lea and Catcheside (1945), in a figure which contained their own data and all other data which had been recorded in earlier literature. At about the same time, a similar curve was published by Demerec and Fano (1944).

The theory, proposed by Muller and Pontecorvo, was that breaks are induced in the chromosomes in mature sperm. After fertilization these breaks are able to rejoin at random and in a proportion of cases the old chromosome configurations are restored. In the other cases, new chromosome configurations are produced,

some of these are vaible and the others are inviable. When one chromosome break is induced, in a mature sperm, it will either restitute or sister chromatid fusion will occur. In the latter case, a dicentric and an acentric will be produced and these will either be lost or act as a primary cause of dominant lethality. In special cases the chromosome loss will leave the cleavage nuclei with viable chromosome complements but in most cases it will cause genetic imbalance and thus dominant lethality. The induction of several chromosome breaks within one nucleus would provide an opportunity for the formation of translocation dicentrics and acentrics and increase the probability that dominant lethality would be caused. Thus it would be expected that the dose-effect curve, for the induction of dominant lethality, would be one-hit at low doses with an increasing multihit component at higher doses.

Additional support for this theory came from comparisons between viable sex chromosome losses and sex-ratio shifts in the progeny of irradiated males.

From the earliest investigations into the genetic effects of radiation (Hanson 1928, Muller 1928), the sex ratio in the progeny of irradiated males has been used as a measure of induced damage. Following the irradiation of rod-X males (Hanson 1928, Muller 1928, Cowen and Gay 1933, Bauer 1942, Demerec and Fano 1944, Lea and Catcheside 1945, Baker 1957) slight sex ratio shifts were induced. However, these were only significant at very high doses. In contrast, significant deficiencies of female progeny were found when ring-X males were irradiated with comparatively low doses (Bauer 1939, 1942, Lea and Catcheside 1945, Baker and von Halle 1955, Baker 1957, Bender 1958).

Viable sex chromosome losses, as measured by the frequencies of XO males, were induced at considerably higher rates in the mature sperm of ring-X males than in the mature sperm of rod-X males (Pontecorvo 1940, Bauer 1942, Luning 1952,

Baker 1957). It was found that viable losses of the ring-X chromosome only accounted for a minor proportion of the induced deficiencies of female progeny, but these two measures of genetic damage followed similar dose-effect curves. From theoretical considerations, it was argued that a break in a ring-X chromosome would have a far higher probability of leading to the formation of a dicentric and thus viable loss. The higher frequencies of sex chromosome losses and the larger sex ratio shifts, in the progeny of irradiated ring-X males, were therefore interpreted as confirming the validity of the chromosome breakage theory of dominant lethality and chromosome loss. By comparing the induced frequencies of viable sex chromosome losses with the deficiencies of females, in the progeny of irradiated ring-X males, it was possible to estimate the probabilities with which a dicentric would cause either viable loss or dominant lethality (Pontecorvo 1940, Lea and Catcheside 1945, Baker 1957).

It was pointed out, by Demerec and Fano (1944), Lea and Catcheside (1945) and Baker (1957), that it was necessary to assume that breaks in the Y chromosome would have as high a probability of causing dominant lethality as breaks in the X chromosome, in order to explain the very small sex ratio shifts found in the progeny of irradiated rod-X males. Baker (1957) argued that, since viability is not greatly affected by the presence or absence of a Y chromosome or fragments of a Y chromosome, it seemed improbable that lethality could be caused by the involvement of a Y chromosome in a bridge-breakage-fusion cycle. This argument, together with several discrepancies in his data, led Baker to suggest that "some novel mechanism might underlie the formation of dominant lethals in sex chromosomes of Drosophila".

4) The approach to the problem:

Differential rates of XO male induction in the different spermatogenic cell

stages, of ring-X males, had only been reported twice in the literature. Lüning (1952) and Sobels (1963) both found that the highest frequencies of XO males were induced in immature germ cell stages. Sobels used a more accurate brood technique than Lüning and found that spermatocytes were the most sensitive stage. However, neither of the above authors recorded the corresponding sex ratio shifts which, according to the theory of Muller and Pontecorvo, were also a measure of the amount of induced chromosome breakage. Therefore it was of primary importance to find out how the sex ratio varied, in relation to the frequencies of XO males, through the brood pattern. The initial experiments were designed to provide comparative dose response curves, for the induction of XO males and sex ratio shift, from the different germ cell stages. Ring-X males were irradiated in nitrogen to ensure consistently uniform conditions and to permit a later investigation into the differential effects of nitrogen and oxygen post-treatments. Another reason for irradiating in nitrogen was that a dose of 3kR in nitrogen was equivalent to a dose of 1kR in oxygen and it was simpler to sub-divide 3kR than 1kR, to obtain the intermediate levels on the dose response curves.

It has been the generally accepted convention that sex ratio is expressed in terms of all the males in the progeny. This convention was initially followed. However, it was later found more convenient to separate the XO males from the normal (XY) males. The induced increases in the frequencies of normal males were then interpreted as a best measure of the amount of differential pre-adult lethality between male and female nygotes. There were several reasons for this re-definition of sex ratio shift. Firstly, evidence was obtained which indicated that there was no strong correlation between the induced frequencies of XO males and the induced sex ratio shifts. This indication of independence was more

pronounced when the sex ratio was expressed in terms of the normal males. Secondly, the term "sex ratio" implied that a measure was being taken of the ratio of X-bearing sperm to Y-bearing sperm, while the available evidence (Pontecorvo 1940, Baker 1957) indicated that a very large proportion of the XO males, in the progeny of irradiated ring-X males, were caused by loss of the X chromosome. Thirdly, XO males and normal males were phenotypically distinguishable classes of progeny and pooling them together made the statistics more complicated than had at first been realised.

MATERIALS and METHODS

1. The stocks used in the present experiments were X^{C2},y B/sc^{8}•Y males and y sc^{S1} In49 sc^{8}; bw; st p^{P} females.

a) The X^{C2} chromosome was isolated by Boche (unpublished) in the progeny of an attached-X female. This chromosome has a stable ring-configuration, with a deficiency for bands 1A1 to 1A3 and a small duplication in region 20, (Schultz and Catcheside 1937).

The X^{C2} chromosome which was used, in the present investigation, carried the recessive marker yellow (y) and the dominant marker bar (B). A peculiarity of this chromosome was that neither X^{C2}, y B/O males nor X^{C2}, y B/X^{C2}, y B females were viable. (See note below).

b) The sc^{8}•Y chromosome was constructed by Muller (1948) and carries the wild alleles of yellow (y) and achete (ac) on the long arm (Y^{L}).

c) The female stock carried the recessive marker yellow (y) on a doubly inverted X chromosome. The X chromosome carried inversion In49 inside inversion sc^{S1} - sc^{8}, to inhibit crossing over. The second chromosome carried the recessive marker brown (bw) and the third chromosome carried the recessive markers scarlet (st) and peach (p^{P}).

Note. The X^{C2}, y B chromosome was checked by Tates (1962, unpublished) and rechecked by the author in 1964. Neural ganglia from third instar male larvae were stained in acetic-orcein and squashed. Ring chromosomes were visible in the cells which contained metaphase figures. The reason for the inviability of X^{C2}, y B/O males and homozygous X^{C2}, y B females is not known.

2. The brood technique:

One-day old X^{C2}, y B/sc^{8}•Y males were treated and the sperm released during the following ten days was sampled in a series of two-day broods, with six females per male per brood. These broods were labelled A (days 1 and 2), B (days 3 and 4), C (days 5 and 6), D (days 7 and 8), and E (days 9 and 10). In most of the experiments brood D was split into two one-day broods, D_1 and D_2, with three females per male per brood. A similar subscript code was used when other two-day broods were split; for example brood C could be split into broods C_1 and C_2. The F_1 progeny were scored on the 14th or 15th day after the start of each brood.

3. The observed F_1 phenotypic (genotypic) classes:

a) normal progeny,

yellow, heterozygous bar females (y/X^{C2}, y B)

non-yellow, round-eyed males (y/sc^{8}•Y)

b) following loss of either the paternal X or Y chromosome, or loss (deletion) of the part of the Y chromosome carrying the wild allele of yellow,

yellow, round-eyed males (y/O or y/sc^{8}(del.)Y)

c) following paternal non-disjunction,

non-yellow, heterozygous bar females (y/X^{C2}, y B/sc^{8}•Y)

yellow, round-eyed males (y/O)

d) following maternal non-disjunction,

non-yellow, round eyed females (y/y/sc^{8}•Y)

yellow, round-eyed females (y/y/O)

e) following early somatic loss of an X chromosome, in a potentially female zygote,

gynandromorphs (y/O: y/X^{C2}, y B or O/X^{C2}, y B: y/X^{C2}, y B)

f) following early somatic loss of a Y chromosome,

mosaic yellow and non-yellow males ($y/sc^{8}.Y$: y/O)

4. The sex-linked lethal test:

To test for sex-linked lethals, normal F_1 females (y/X^{C2}, y B) were individually mated to their non-yellow, round-eyed brothers. The F_2 cultures were then scored for the presence or absence of X^{C2}, y B males. An F_2 culture was scored as a lethal when it contained more than 15 flies and none of these were X^{C2}, y B males. When less than 15 flies were present in any culture and none of these was an X^{C2}, y B male, the irradiated chromosome was retested by individually mating F_2 y/X^{C2}, y B females to their brothers and then scoring for the presence or absence of X^{C2}, y B males in the F_3 cultures.

5. The irradiation and gas treatments:

In all experiments, the irradiations were given with an ENRAF machine set at 100kV and with 1mm Al filtration. For the high dose rate (2,600R/min) the irradiation chamber was placed as close as possible to the radiation source and the ENRAF machine was set at 4mA. To obtain a lower dose rate (400R/min), the source to target distance was increased and the ENRAF machine was set at 2mA. The irradiations were measured on a Philips dosimeter.

For irradiation, males were placed in a shallow perspex irradiation chamber. A pre-treatment of purified nitrogen was then given for 25 minutes, at a rate of 1 litre per minute. The purpose of this pre-treatment was to ensure that the males were saturated with nitrogen during the time of irradiation. Post-treatments, in either purified nitrogen or in oxygen, were given for 25 minutes at a rate of one litre per minute. The commercial nitrogen was purified by passing it through three washing flasks, with sintered glass bottoms, which contained "O_2-multirapid" (Unionapparate Baugesellschaft M.B.H., Karlsruhe).

RESULTS: 1

Dose response curves:

The primary purpose of the first two groups of experiments was to find a suitable dose level for use in further experiments. These experiments were also designed to confirm that the sex-linked lethal and XO males peaks occurred in different broods. The third object of these experiments was to find out how the frequencies of normal males varied through the brood pattern.

Six nitrogen pre- and post-treatment experiments, with doses ranging from 0kR to 3kR, provided the data for the dose response curves. In the first group of experiments (I, II and III) doses of 3kR, 2kR and 1kR were used. The second group (IV, V and VI) contained doses of 1kR, 0.5kR, and a nitrogen-only control. A basic two-day brood pattern was used, with six females per male per brood. In the second group of experiments, for a reason which will be given later, the fourth brood was split into two one-day broods.

Sex-linked lethals:

The brood patterns and frequencies of sex-linked lethals were of secondary importance, because many sex-linked lethal tests have been carried out by Professor Sobels using the same stocks and brood sequence. Therefore, to allow the maximum amount of time for scoring the F_1, sex-linked lethal tests were restricted to broods A, C, D and E of the first group of experiments. The data which were obtained from these tests are given in the table below.

Table 1:1

Brood	A	C	D	E
Experiment				
I(3kR)	4.48 ± 0.80 (30/670)	9.65 ± 1.01 (83/860)	11.46 ± 2.00 (29/253)	1.53 ± 0.46 (11/517)
II(2kR)	3.15 ± 0.66 (22/698)	6.02 ± 0.82 (51/847)	3.75 ± 0.75 (24/640)	1.79 ± 0.51 (12/670)
III(1kR)	0.92 ± 0.46 (4/464)	3.82 ± 0.86 (19/498)	2.90 ± 0.74 (15/517)	0.96 ± 0.55 (3/314)

From the horizontal rows of table 1:1 it can be seen that in each experiment there was a significant increase in the frequencies of sex-linked lethals from brood A to brood C. In experiments II and III the frequencies then decreased through brood D to brood E, thus following a pattern consistent with that which had been found in the preliminary experiments (see fig 1). In experiment I there was very high sterility in brood D and this is reflected by the low number of chromosomes which were available for testing and the consequently very high standard error. Thus, the brood pattern in experiment I was not significantly different from that which was found in experiments II and III.

In the vertical columns of table 1:1 it can be seen that in each brood, except E, the induced frequencies of sex-linked lethals were dose dependent. In the older germ cell stages, broods A and C, the frequencies of XO males increased linearly with dose.

For the subsequent comparison with the F_1 data, the most important points were that

a) at all three dose levels the frequencies of sex-linked lethals increase significantly from brood A to brood C, and

b) there was a tendency for the frequencies of sex-linked lethals decline from brood C to brood D.

It is possible that the highest frequencies of sex-linked lethals were induced in the germ cells sampled in brood B. However, this would be inconsistent with the results of other experiments.

The F_1.

The control experiment VI:

For reference purposes, all the broods of the control experiment were pooled and the total data are shown below:-

Table 1:2

♂♂	♀♀	n.	♂/n.%	XO♂♂	%	XXY♀♀	%
19,833	17,128	37,079	53.5	118	0.32	20	0.05

There was no heterogeneity between broods, with respect to either the frequencies of normal males (χ^2 = 4.4, df.5, P>0.05) or the frequencies of XO males (χ^2 = 2.8, df.5, P>0.05). The frequency of normal males was significantly higher than the theoretically expected 50% (P<0.01), but was very close to the frequency of normal males which Baker and von Halle (1955) found in the progeny of X^{Cl} males carrying the sc^8·Y chromosome.

Experiments I, II, III, IV and V.

The data obtained from the F_1 of the first and second groups of experiments are given in tables 1:3 and 1:4 respectively. At each dose level, the frequencies of XO males were uniform through the first three broods and this can be seen in the similarity between the XO males dose response curves in these

three broods (fig. 1:1, broods A, B and C). In experiments I and II (table 1:3) there was a marked XO males peak in brood D. No similar peak was evident in experiment III and it was therefore decided to split brood D into two one-day broods, in the second group of experiment. However, even when brood D was split (table 1:4) no XO male peak was induced at the lower dose levels. In all experiments the frequencies of XO males were low in brood E. Thus the pattern which emerged from the XO male data was, with doses of 1kR and less the frequencies of XO males were dose dependent but showed very little between-brood variation, while at higher doses there was a marked XO male peak in brood D.

The within brood dose response curves for the frequencies of normal males are shown in figure 1:2. From this figure it can be seen that increases in the frequencies of normal males were induced in broods A, B and C, while in broods D and E there was a tendency for the frequencies of normal males to decline with dose. The frequencies of normal males did not show the same uniformity in the first three broods, as the frequencies of XO males.

There were three main parameters of radiation damage, sex-linked lethals, XO males, and normal male frequencies. Each of these three parameters showed a different brood pattern, the frequencies of normal males even showing different brood patterns in different experiments.

Relatively low numbers of exceptional females (XXY) were found. Therefore the data from experiments I, II and III were pooled and the totals are shown below, together with the corresponding XO male totals and the XXY/XO ratios.

Table 1:5

Brood	A	B	C	D	E
XXY	4	6	6	11	13
XO	274	377	440	221	110
XXY/XO	0.015	0.016	0.014	0.050	0.118

In table 1:5 it can be seen that all of the XXY/XO ratios are relatively low. the uniformity in the first three broods was caused by the fact that the induced frequencies of XO males were uniform and the XXY females were all caused by non-disjunction which occurred prior to irradiation. The increase in the XXY/XO ratio in brood D indicates that irradiated spermatocytes were being sampled in this brood. The frequencies of minor classes of progeny, such as XXY females and gynandromorphs, will be considered in more detail when the main results of the following irradiation experiments have been described.

Table 1:3 The frequencies of normal males, XO males, XXY females, and gynandromorphs in experiments I (N_2.3kR.N_2), II ($N_2$2kR.N_2), and III ($N_2$1kR.N_2)

Brood	Expt.	♂♂	♀♀	n.	♂%	XO♂	%	XXY	%	Gyn.	%
A	I	2687	1874	4717	57.0	156	3.31	1	0.02	12	0.25
	II	2394	2066	4549	52.6	89	1.96	0	0	2	0.04
	III	1130	1012	2171	52.0	29	1.34	3	0.14	0	0
B	I	3495	2615	6302	55.5	192	3.05	1	0.02	5	0.08
	II	4239	3343	7706	55.0	124	1.61	4	0.05	3	0.04
	III	3130	2577	5768	54.3	61	1.06	1	0.02	1	0.02
C	I	2561	1745	4471	57.3	165	3.69	3	0.07	6	0.13
	II	4163	2889	7259	57.3	207	2.85	3	0.04	8	0.11
	III	2351	1921	4340	54.2	68	1.57	0	0	5	0.12
D	I	421	313	805	52.3	71	8.82	2	0.25	1	0.12
	II	1172	961	2256	52.0	123	5.45	8	0.35	0	0
	III	969	829	1825	53.1	27	1.47	1	0.06	1	0.06
E	I	1952	1757	3786	51.6	77	2.03	8	0.211	1	0.03
	II	1918	1747	3697	51.9	32	0.85	3	0.08	0	0
	III	642	571	1214	52.9	1	0.08	2	0.17	1	0.08

Table 1:4 The frequencies of normal males, XO males, XXY females, and gynandromorphs in experiments IV (N_2.1kR.N_2), V ($N_2$0.5kR.N_2), and VI (N_2.0kR.N_2).

Brood	Expt.	♂♂	♀♀	n.	♂/n%	XO♂	%	XXY	%	Gyn.	%
A	IV	3629	2956	6697	54.2	112	1.67	4	0.06	17	0.25
	V	3163	2874	6099	51.9	62	1.02	7	0.11	12	0.20
	VI	4571	3807	8408	54.4	30	0.36	1	0.01	13	0.15
B	IV	3879	3324	7308	53.1	105	1.44	3	0.04	16	0.22
	V	3894	3191	7136	54.6	51	0.71	4	0.06	17	0.24
	VI	4982	4330	9345	53.3	33	0.35	7	0.08	15	0.16
C	IV	3552	2920	6556	54.2	84	1.28	0	0	17	0.26
	V	3637	3082	6788	53.6	69	1.02	3	0.04	10	0.15
	VI	3861	3441	7323	52.7	21	0.29	4	0.05	12	0.16
D_1	IV	1063	935	2049	51.9	51	2.5	2	0.10	4	0.20
	V	1206	1050	2282	52.8	26	1.14	1	0.04	7	0.31
	VI	2156	1863	4031	53.5	12	0.30	3	0.07	10	0.25
D_2	IV	1224	1082	2348	52.1	42	1.79	1	0.04	7	0.30
	V	845	740	1601	52.8	16	0.99	2	0.12	4	0.25
	VI	1230	1061	2300	53.5	9	0.39	2	0.08	7	0.30
E	IV	1793	1513	3356	53.4	50	1.49	4	0.12	11	0.33
	V	1889	1612	3524	53.6	23	0.65	0	0	9	0.26
	VI	3033	2626	5672	53.5	13	0.23	3	0.05	10	0.18

Figure 1:1. The dose response curves of XO male frequency, in the two-day brood periods A, B, C, D, and E, (the data are given in tables 1:3 and 1:4). This figure shows the similarity between the dose response curves in broods A, B, and C, and the considerably steeper dose response curve in the spermatocyte brood (D).

Fig. 1 : 1

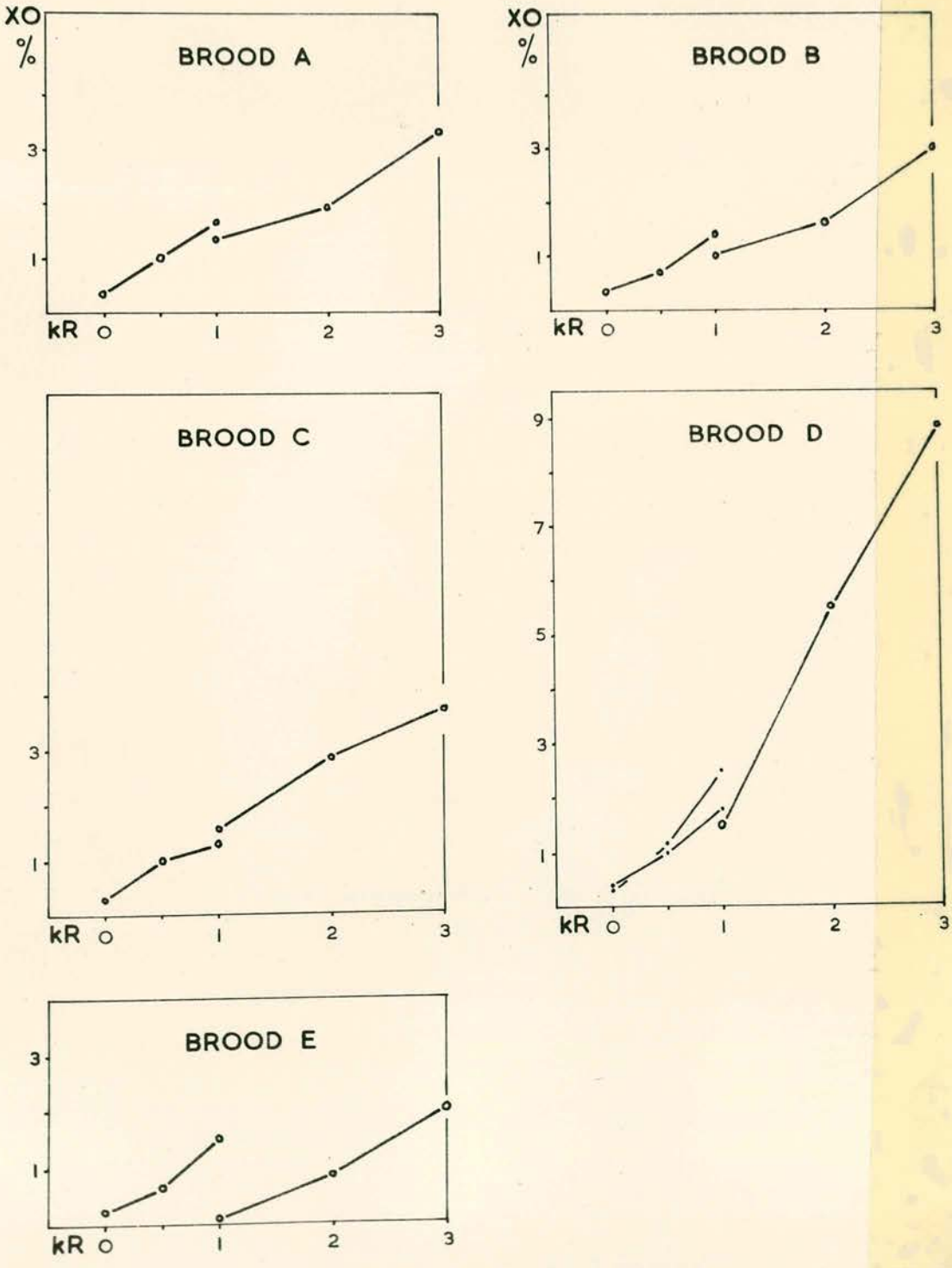
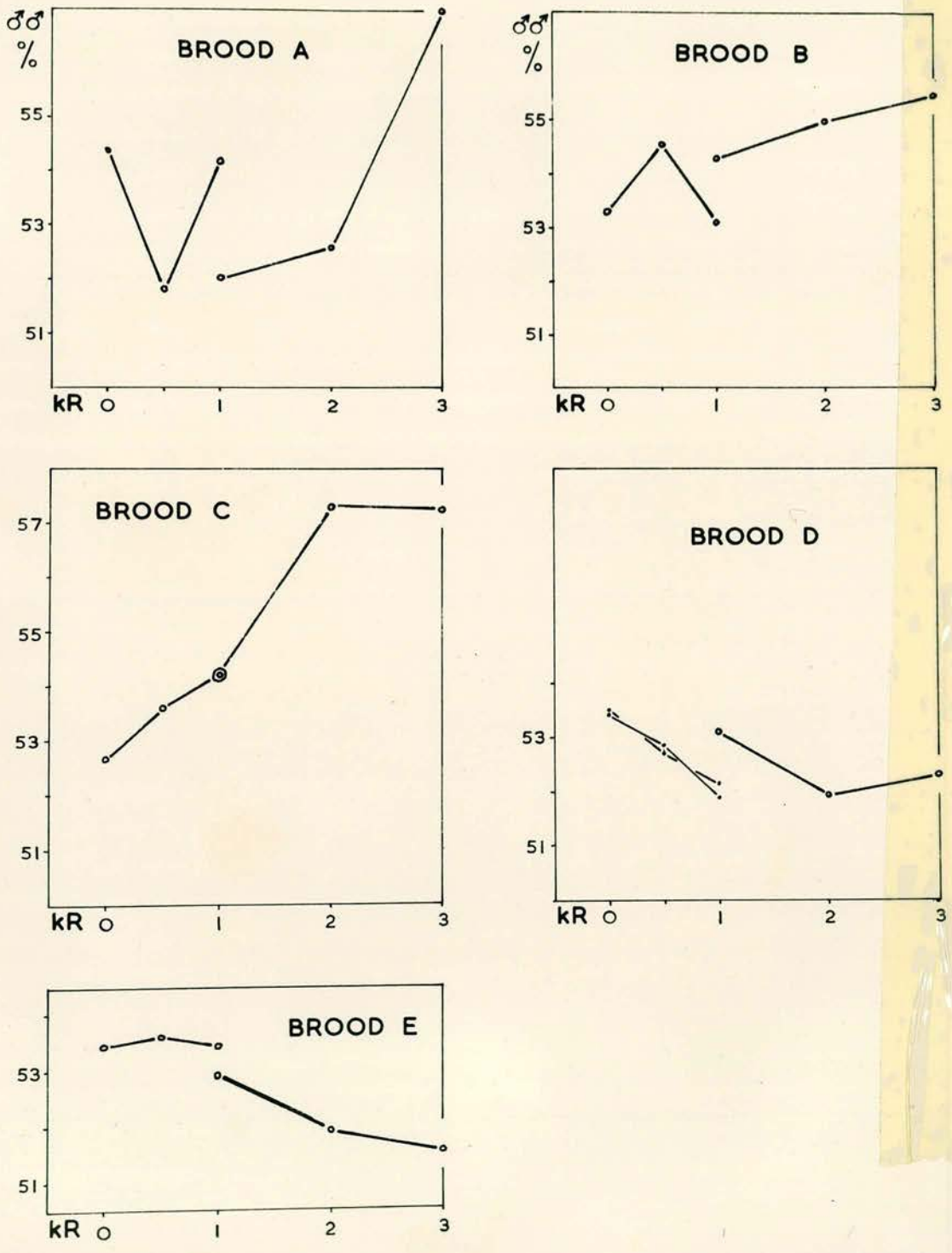

XO %
BROOD A
3
1
kR 0 1 2 3
XO %
BROOD B
3
1
kR 0 1 2 3
BROOD C
3
1
kR 0 1 2 3
BROOD D
9
7
5
3
1
kR 0 1 2 3
BROOD E
3
1
kR 0 1 2 3

Figure 1:2. The dose response curves of normal male frequency, in the two-day brood periods A, B, C, D, and E, (the data are given in tables 1:3 and 1:4). This figure shows the irregularity of the normal male dose response curves, particularly in brood A. A tendency for the frequency of normal males to decrease with dose is shown in broods D and E.

Fig. 1 : 2


♂♂ %
BROOD A
55
53
51
kR 0 1 2 3
♂♂ %
BROOD B
55
53
51
kR 0 1 2 3
BROOD C
57
55
53
51
kR 0 1 2 3
BROOD D
53
51
kR 0 1 2 3
BROOD E
53
51
kR 0 1 2 3

Discussion:

Experiments I to VI were designed to provide a basis for further investigation, firstly by indicating an optimum dose level, secondly by confirming that the highest frequencies of XO males occurred in a later brood than the highest frequencies of sex-linked lethals, and thirdly by showing how the frequencies of normal males varied through the brood pattern.

Establishment of the optimum dose level was dependent upon two properties, a) that it would permit fertility throughout the brood sampling period, and b) that it would induce significant sex-linked lethal and XO male peaks. Fertility was maintained throughout the brood period in all experiments, but significant XO male peaks were only induced by doses of 2kR (experiment II) and 3kR (experiment I). Therefore, it was concluded that further experiments would require doses of either 2kR or 3kR. In both experiments I and II, the XO male peak was restricted to brood D while high frequencies of sex-linked lethals were found in brood C. Because of the low number of tested chromosomes, the sex-linked lethal peak in experiment I extended from brood C into brood D, but this was inconsistent with the patterns which were obtained in experiments II and III, and the preliminary experiments (figs. 1 and 2). From the distributing of these two peaks, the decrease in fertility in brood D at higher dose levels, and the increase of the XXY/XO ratio in brood D, it was provisionally concluded that broods C and D represented irradiated spermatids and spermatocytes, respectively.

The third object of the first two groups of experiments was to investigate the brood pattern changes of the frequencies of normal males. This measure of radiation damage did not present as consistent a picture, either within or between broods, as did the frequencies of sex-linked lethals and XO males.

The normal male dose-response curve in brood A, which presumably represented mature sperm, was the most difficult to interpret. Earlier investigators have consistently found that the increase in the proportion of male progeny, obtained after the irradiation of mature sperm, were greater than could be accounted for by the conversion of potential female zygotes (XX) into XO males. Indeed, this observation was the basis of the hypothesis that breakage of the ring-X chromosome causes both "viable" and "inviable" losses. The latter type of loss was assumed to act as a dominant lethal and cause a preferrential elimination of potential female zygotes. The normal male dose-response curve, in brood A of the present experiments (fig. 1:2) did not show a marked linear increase with dose. The frequency of normal males in brood A of the control experiment was slightly higher than the average control frequency and therefore, to avoid bias, the frequencies of normal males in brood A of the irradiation experiments were compared to the average control frequency (53.5%). This comparison is given in the table (1:6) below.

Table 1:6

Expt.(dose)	I (3kR)	II (2kR)	III (1kR)	IV (1kR)	V (0.5kR)
Frequency of normal ♂♂	57.0 ± 0.7	52.6 ± 0.7	52.0 ± 1.1	54.2 ± 0.6	51.9 ± 0.6
Deviation from 53.5%	+ 3.5	- 0.9	- 1.5	+ 0.7	- 1.6
χ^2	11.7	0.6	0.8	0.7	3.0
P	<0.01	>0.05	>0.05	>0.05	>0.05

In experiments II, III, IV and V, the frequencies of normal males in brood A did not differ significantly from the control frequency. In three of these experiments the frequencies tended to be lower than control. Therefore, it was difficult to interpret the data as being consistent with the finding of other investigators. On the other hand, the frequency of normal males in brood A of experiment I was significantly higher than control, and, while it appeared to be inconsistent with the data of experiments II to V, it was consistent with the published data of other authors. Thus, in brood A, the frequencies of XO males increased linearly with dose (fig. 1:1), but there was no correspondingly linear increase of the frequency of normal males (fig.1:2). In other words, the frequencies of XO males were typical of those obtained after the irradiation of the mature sperm of ring-X males while the frequencies of normal males were atypical. This indicated that the assumption of a correlation between the frequencies of XO males, "viable" loss, and the frequencies of normal males, "inviable" loss, was possibly an oversimplification.

The normal male dose response curves in broods B and C (fig. 1:2) both showed an increase with dose and in these broods the data could be interpreted as indicating that there had been a preferential lethality affecting female zygotes. In broods D and E the frequencies of normal males tended to decrease with dose. This indicated that losses of the Y chromosome were contributing to the production of XO males. In brood D, where there were high frequencies of XO males, it seemed reasonable to assume that a small proportion of these XO males would have been caused either by breakage of the Y chromosome, or its involvement in non-disjunction or crossing-over. However, in brood E of experiments I and II the decreases of the frequencies of normal males were large enough to indicate that loss of the Y chromosome had been the sole cause of XO male production. This appeared to be an improbably conclusion, although it fitted the data, as is shown below in table 1:7.

Table 1:7

Experiment	I	II
Frequency of normal males in brood E	51.6 ± 0.8	51.9 ± 0.8
Deviation from 53.5%	- 1.9	- 1.4
Frequency of XO males in brood E	2.03	0.85

In summary, it was concluded from the first six experiments that doses of 2kR and 3kR were necessary for the induction of a marked XO male peak. Later experiments indicated that 2kR was the optimum dose. The peak of XO males occurred in brood D and this confirmed the pattern which had been found in the preliminary experiments. The frequencies of normal males tended to show a peak in brood C and there were consistently low frequencies in broods D and E. However, there were irregularities in the normal male dose response curves, particularly in brood A.

2A. Experiments VII, VIII and IX

Since the proposed mechanisms of ring-X chromosome breakage and loss (Bauer 1942, Pontecorvo 1941, Muller 1940 and Baker 1957) assume a correlation between the frequencies of XO males and the increases of the frequencies of normal males, further experiments were devoted to an analysis of this correlation. The first group of these experiments was designed to investigate two questions, whether oxygen post-treatment would modify either the frequencies of XO males or normal males, or both, and secondly whether splitting brood D into two one-day broods, at the higher dose levels, would allow a finer analysis of the XO male peak.

Brood D was characterised in four ways. It contained the XO male peak, low freqnencies of normal males, a relatively high XXY/XO ratio, and the lowest number of progeny. While the splitting of brood D in experiments IV, V and VI had provided no useful information this was probably because the doses used in these experiments had been too low.

To investigate the differential effects of oxygen and nitrogen post-treatments it was desirable to use the highest possible dose. Therefore, experiments VII and VIII were carried out with a dose of 3kR and with oxygen and nitrogen post-treatments, respectively. It was considered possible that the high dose level might cause sterility in either of the one-day broods and therefore experiment IX was carried out with a dose of 2kR and with a nitrogen post-treatment. The data obtained in these three experiments are given in table 2:1.

In all three experiments, the frequencies of XO males were uniform in the first three broods, with a slight but not significant dip in brood B. The XO male peak occurred in brood D_1 and in experiment IX this peak continued

into brood D_2. Low frequencies of XO males were found in brood E. In all three experiments the frequencies of XO males showed the same brood pattern and this was consistent with the pattern which had been found in earlier experiments.

The frequencies of normal males tended to show a peak in brood C and were low in broods D_1, D_2 and E. In all three experiments the frequencies of normal males were relatively high in brood A and the patterns were thus consistent with the pattern which had been found in experiment I.

At the higher dose level there was almost complete sterility in brood D_2 which indicated that a dose of 3kR was too high to be used in further experiments. In all later experiments a dose of 2kR was used. The coincidence of the high sterility periods indicated that the rate of sperm utilization had not been differentially affected by the two post-treatments.

Post-treatments, oxygen versus nitrogen (experiments VII and VIII)

The XO male and normal male brood patterns, of experiments VII and VIII are illustrated in figures 2:1a and 2:1b. In these two figures it can be seen that the oxygen post-treatment enhanced the frequencies of XO male in all broods except E, while the nitrogen post-treatment enhanced the frequencies of normal males in all broods. The reality of these post-treatment effects was tested again in later experiments, because the frequencies of XO males and normal males in experiment VII were very similar to the frequencies which had been found in experiment I (table 1:2), where a nitrogen post-treatment had been given.

Dose-effect, with nitrogen post-treatments (experiments VIII and IX)

A comparison between the data obtained in experiments VIII and IX (figures 2:2a and 2:2b) revealed that there had been no dose effect on the

frequencies of normal males. Curiously enough, a similar discrepancy had been found between the data of experiments II and II, but in that case the frequencies of XO males had shown a dose effect response while there had been no dose effect on the frequencies of normal males.

Conclusion

These experiments confirmed the brood pattern of XO male frequency and indicated that the general brood pattern of normal male frequency was similar to that which had been found in experiment I. In each experiment the period of highest sterility occurred on the eighth day of sperm sampling and in experiment IX this coincided with the highest frequency of XO males. It was concluded that a narrow range of germ cells were particularly sensitive to either the induction of dominant lethality or killing by radiation and that these same germ cells were also the most sensitive to the induction of chromosome loss. An attempt was made to obtain a more direct sampling of these sensitive cells, by irradiating pupae. However, this led to the discovery of a new phenomenon and will be described later.

Oxygen post-treatment appeared to increase the frequencies of XO males and decrease the normal male frequencies. This observation and the different brood patterns for XO males on the one hand and normal male frequencies on the other, as well as the dose effect discrepancies, indicated that either independent mechanisms were responsible for the induced frequencies of XO males and the increases in the frequencies of normal males or experimental conditions were able to modify independently these two parameters of radiation damage. The next experiments were designed to distinguish between these two possibilities.

Table 2:1 The frequencies of normal males, XO males, XXY females, and gynandromorphs in experiments VII (N_2.3kR.O_2), VIII (N_2.3kR.N_2), and IX (N_2.2kR.N_2).

Brood	Expt.	♂♂	♀♀	n.	♂/n%	XO♂	%	XXY	%	Gyn.	%
A	VII	2130	1402	3655	58.3	123	3.37	2	0.05	10	0.27
	VIII	2580	1551	4242	60.8	111	2.62	1	0.02	7	0.17
	IX	2467	1624	4197	58.8	106	2.53	2	0.05	8	0.19
B	VII	1798	1269	3150	57.1	83	2.63	0	0	7	0.22
	VIII	2202	1340	3613	60.9	71	1.97	5	0.13	2	0.06
	IX	2471	1794	4332	57.0	67	1.55	2	0.05	4	0.09
C	VII	1140	651	1857	61.4	66	3.55	0	0	3	0.16
	VIII	1470	804	2332	63.0	58	2.49	3	0.13	1	0.04
	IX	1880	1222	3177	59.2	75	2.36	3	0.09	1	0.03
D_1	VII	212	179	428	49.5	37	8.64	1	0.23	1	0.23
	VIII	62	40	108	57.4	6	5.55	0	0	0	0
	IX	304	228	559	54.4	27	4.83	3	0.55	0	0
D_2	VII	7	8	19	-	4	-	0	-	0	-
	VIII	6	11	18	-	1	-	0	-	0	-
	IX	92	67	176	52.3	17	9.66	0	0	1	0.57
E	VII	2270	2012	4344	52.3	62	1.43	3	0.07	0	0
	VIII	858	616	1508	56.9	34	2.25	1	0.07	1	0.07
	IX	1719	1382	3137	54.8	36	1.15	0	0	1	0.03

Figure 2:1. The brood patterns for (a) XO male frequency and (b) normal male frequency in experiments VII (+ — —+), $N_2 \cdot 3kR \cdot O_2$, and VIII (x———x), $N_2 \cdot 3kR \cdot N_2$.

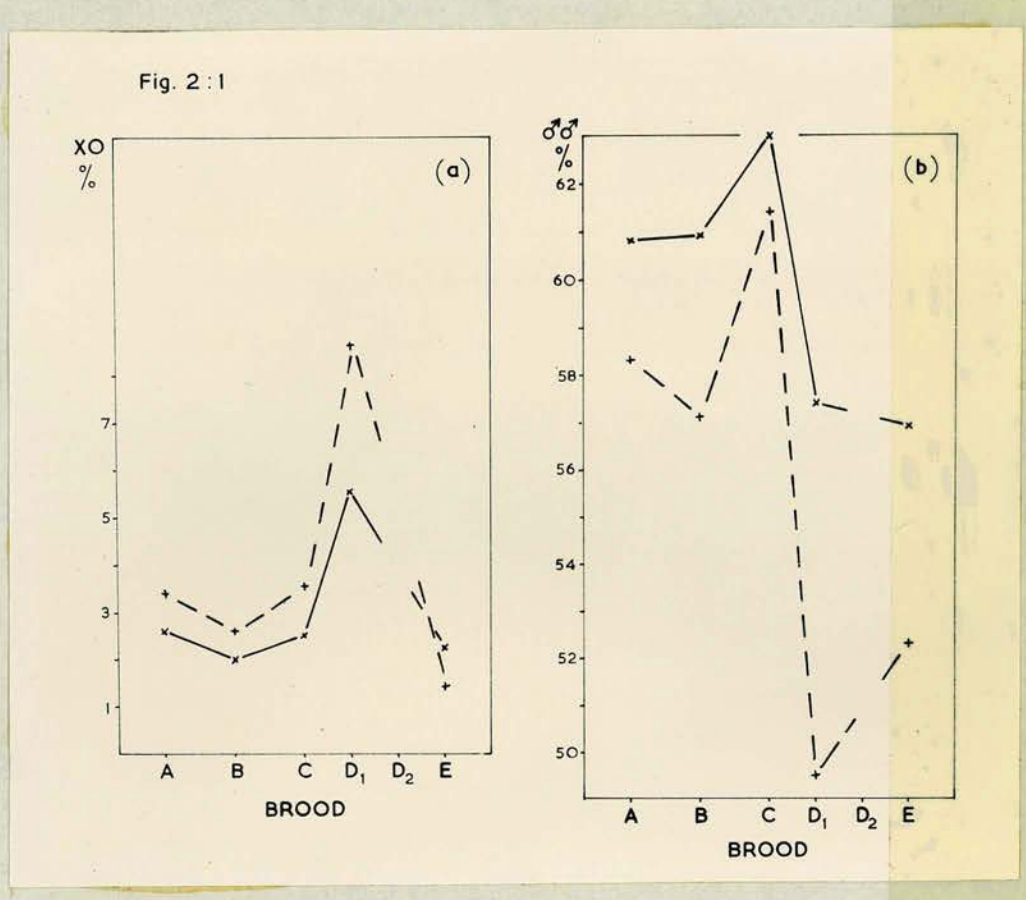
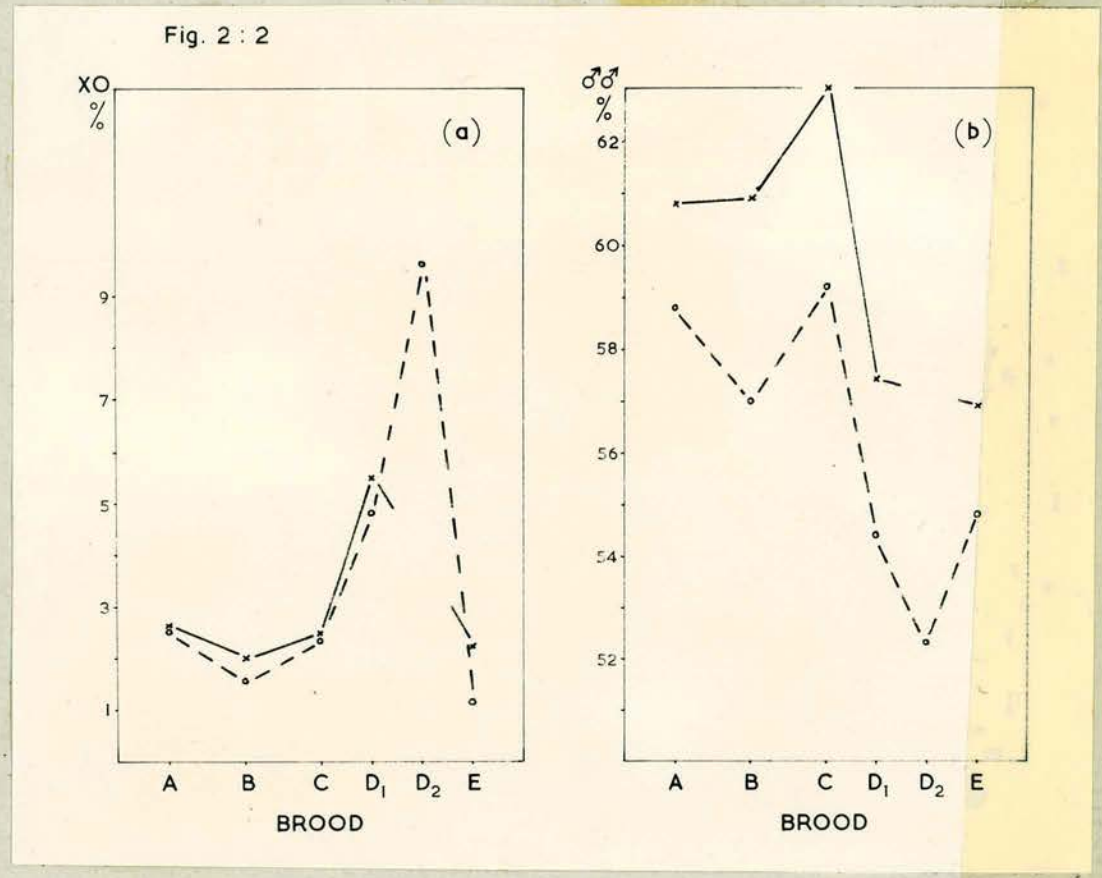


Figure 2:2. The brood patterns for (a) XO male frequency and (b) normal male frequents in experiments VIII (x———x), N_2.3kR.N_2, and XI (o— — —o), N_2.2kR.N_2.



Results: 2b, experiments X, XI and XII

"O_2-multirapid" and oxygen post-treatment

In all of the foregoing experiments, a note had been kept of the time of death of each of the irradiated males. When these data were examined, it was found that there had been a relatively high mortality within 48 hours after radiation and nitrogen treatment. This is shown in table 2:2 below.

Table 2:2

Experiment	Dose	Number of X^{C2} males treated	Number of males dead within 48 hrs.	Percentage mortality
I	3kR	77	12	15.6
II	2kR	62	16	25.8
III	1kR	41	19	46.3
IV	1kR	60	24	40.0
V	0.5kR	60	25	41.7
VI	0kR	50	17	34.0
VII	3kR ($.O_2$)	60	2	3.3
VIII	3kR	70	23	32.8
IX	2kR	70	25	35.7

Two experiments in the above table were particularly significant. Firstly the mortality in the nitrogen-only control experiment (VI) was as high as in the other nitrogen post-treated experiments and this indicated that the mortality was caused by exposure of the males to the purified nitrogen. Secondly, when an oxygen post-treatment was given (experiment VII) the early mortality was practically eliminated. This second observation indicated that

a specific component of the nitrogen treatment was responsible for the killing of the irradiated males. The "O_2-multirapid"solution, which was used to remove all traces of oxygen from the commercial nitrogen, was known to be toxic. It was therefore suspected that the stream of nitrogen gas had caused some of the "O_2-multirapid" to vaporise and that this had contaminated the purified nitrogen. The males in the irradiation chamber would have adsorbed some of the contaminating "O_2-multirapid". The oxygen post-treatment then saturated the adsorbed "O_2-multirapid" and thus rendered it harmless.

To test this interpretation, a wash bottle was filled with glass wool and inserted between the ultimate "O_2-multirapid" wash bottle and the irradiation chamber. In a pilot control experiment it was found that the insertion of the glass wool filter eliminated the lethal affect of the nitrogen treatment.

The importance of the argument given above was that the "O_2-multirapid" adsorbed by the irradiated males in earlier experiments could have been the cause of some of the discrepancies in the F_1 data. It has been shown, by Sobels (1955) and Clark (1956) that other toxic agents, such as cyanide or azide, can modify the genetic effects of radiation when they are given as a co-treatment. Since the amount of multirapid which was absorbed by the treated males would have depended on many factors, such as room temperature, the length of tubing between the ultimate "O_2-multirapid" bottle and the irradiation chamber, minor variations in the rate of nitrogen flow, and the distribution of the males within the irradiation chamber, it seemed probable that this might be the cause of discrepancies between experiments. This possibility was tested by carrying out two 2kR nitrogen pre- and post-treatment experiments, which differed only by the presence or absence of the glass wool

filter between the "O_2-multirapid" bottles and the irradiation chamber. These two experiments were designated X and XII respectively. At the same time, in experiment XI, the effects of an oxygen post-treatment were re-investigated. In experiment XI the pre-treatment nitrogen was passed through the glass wool filter. This was to ensure against the possibility that there might be an interaction between an "O_2-multirapid" effect and an oxygen post-treatment effect.

The early mortality rates in each experiment are shown below.

Table 2:3

Experiment	dose	Number of males irrad.	Males dead within 48 hrs.	Percentage mortality
X	2kR	60	6	10
XI	2kR (.O_2)	60	0	0
XII	2kR	71	15	21

From table 2:3 it can be seen that the oxygen post-treatment (experiment XI) again counteracted the cause of the early mortality of the treated males. However, the mortality in experiment X indicated that the glass wool filter had not completely removed the "O_2-multirapid" from the nitrogen. The differential effects of the above treatments, on the amount of induced genetic damage, will be described below. Because of the partial failure of the glass wool filter, several other methods of eliminating "O_2-multirapid" from the nitrogen were considered. However, none of these were found to be satisfactory and in all later experiments the original set-up was used, with three "O_2-multirapid" wash bottles between the nitrogen supply and the irradiation chamber.

The F_1 :

The data obtained in the F_1 of experiments X, XI and XII are given in table 2:4. In all three experiments the frequencies of XO males were uniform through the first three broods, then there was a peak in broods D_1 and D_2, followed by low frequencies in brood E. This confirmed the brood patterns which had been found in earlier experiments. Two minor details distinguished experiments X and XI; the XO male frequencies did not dip in brood B and the highest frequencies were found in brood D. Neither the use of a glass wool filter nor the oxygen post-treatment caused a significant modification of the frequencies of XO males.

On the other hand, the frequencies of normal males were affected by both treatment modifications. Following oxygen post-treatment, the frequencies of normal males showed a slight tendency to increase from brood A to brood B to brood C. In the two nitrogen post-treatment experiments the frequencies of normal males showed a significant peak in brood B. In each of broods A, B and C the frequencies of normal males in experiment XII were significantly higher than the corresponding frequencies in experiment X ($\chi^2_A = 3.9$: $\chi^2_B = 5.4$: $\chi^2_C = 4.1$, each χ^2 with 1 df.).

In all three experiments the frequencies of normal males decreased markedly from brood C to brood D_1, and then remained low in broods D_2 and E. The absence of significant increases in the frequencies of normal males in the last three broods was consistent with the brood patterns found in earlier experiments.

Conclusion:

From these three experiments it was concluded that the frequencies of normal males could be modified by changes of the experimental conditions which did not affect the frequencies of XO males. "O_2-multirapid" contamination of the nitrogen caused an enhancement of the frequencies of normal males without changing the brood pattern, while oxygen-post treatment caused the frequencies of normal males to be uniform through the first three broods. This indicated that the two treatment variations were modifying different components of the mechanism causing the increase in the frequencies of normal males. From this it followed that there were probably several causes of the dominant lethality which preferentially eliminated female zygotes.

Table 2:4 The frequencies of normal males, XO males, XXY females and gynandromorphs in experiments X (N_2.2kR.N_2) and XI (N_2.2kR.O_2), in both of these experiments the purified nitrogen was passed through a glass wool filter, but not in experiment XII (N_2.2kR.N_2).

Brood	Expt.	♂♂	♀♀	n.	♂/n%	XO♂	%	XXY	%	Gyn.	%
A	X	2935	2170	5247	55.9	142	2.7	0	0	11	0.12
	XI	3197	2304	5656	56.5	155	2.74	6	0.11	18	0.32
	XII	3351	2281	5796	57.8	164	2.83	5	0.09	16	0.28
B	X	3149	2081	5369	58.7	139	2.59	3	0.06	4	0.07
	XI	3233	2286	5679	56.9	160	2.82	3	0.05	7	0.12
	XII	3504	2139	5761	60.8	118	2.05	2	0.03	5	0.09
C	X	1883	1393	3362	56.0	86	2.56	1	0.03	4	0.12
	XI	1997	1382	3485	57.3	106	3.04	4	0.11	3	0.09
	XII	1861	1218	3181	58.5	102	3.2	2	0.06	4	0.13
D_1	X	729	569	1372	53.1	74	5.39	3	0.22	2	0.15
	XI	825	634	1569	52.6	110	7.01	2	0.13	1	0.06
	XII	761	593	1453	52.4	99	6.81	5	0.34	2	0.14
D_2	X	176	131	322	54.7	15	4.66	0	0	1	0.13
	XI	459	340	848	54.1	49	5.78	5	0.59	4	0.47
	XII	154	116	293	52.6	23	7.85	1	0.34	0	0
E	X	1410	1100	2556	55.2	46	1.80	2	0.08	3	0.12
	XI	2224	1738	4034	55.1	72	2.37	1	0.02	4	0.10
	XII	1709	1428	3200	53.4	63	1.97	3	0.09	4	0.13

Results: 2c, experiments XIII and XIV

In the previous group of experiments, X, XI and XII, it was shown that the frequencies of normal males could be modified by varying the experimental conditions. However, this did not exclude the possibility that post-fertilization conditions might also be causing a modification of the frequencies of normal males. To test this latter possibility, broods A and C were each split into two one-day broods. Then, if post-fertilization conditions were causing a modification of the frequencies of normal males, it would be expected that the differences between consecutive one-day broods would be as great as the differences which had been found between consecutive two-day broods in earlier experiments.

At the same time, an oxygen post-treatment experiment was also carried out, using an identical brood sequence. The purpose of this experiment was to further investigate the conclusion that such a treatment either eliminated or suppressed a portion of the differential dominant lethality.

These two experiments were numbered XIII, with an oxygen post-treatment, and XIV, with a nitrogen post-treatment. In each experiment broods A, C, D and E were split into one-day broods. The data obtained from these two experiments are given in table 2:5 and the brood pattern of XO males and normal males are shown in figures 2:3a and 2:3b respectively. In figure 2:3a it can be seen that the frequencies of XO males remained at the same level from brood A_1 to brood C_2 and that there was no differential effect of the two post-treatment in these five broods. The XO male peak was relatively low and occurred in broods D_2 and E_1. This is the pattern which would have been expected if the greater number of one-day broods had

caused a slowing down of the rate of sperm utilization. In the last three broods there was a tendency for the nitrogen post-treatment to cause a relative enhancement of the frequencies of normal males, but this was not significant.

The frequencies of normal males, in both experiments (fig. 2:3b), tended to decrease from brood A_1 to brood D_2. In experiment XIV this decrease occurred in a step-wise manner, as would have been expected if the expression of a proportion of the differential dominant lethality was dependent upon unknown post-fertilization conditions. In experiment XIII the frequencies of normal males decreased linearly from brood A_1 to brood D_2 and this was interpreted as indicating that the proportion of the dominant lethality which was suppressed by an oxygen post-treatment corresponded to the proportion of dominant lethality whose expression was dependent upon the unknown post-fertilization conditions.

A decrease in the frequencies of normal males, through the first six days of sperm sampling, had not been found in any of the experiments in which broods A, B and C had each been two-day broods. It therefore seemed probable that the more frequent brood changing had caused a modification of the basic normal male brood pattern and this could not be simply accounted for by a slowing down of the rate of sperm utilization.

Conclusion:

From these experiments it was concluded that the radiation induced increases in the frequencies of normal males resulted from a combination of several causes. This was the only explanation which was consistent with the observed fact that a change in any of the experimental protocols caused a modification of the frequencies of normal males. In contrast, the induced frequencies of XO males were regular and this confirmed the earlier indication that the assumption of a correlation between the induced frequencies of XO males and the increases in the frequencies of normal males was an oversimplification.

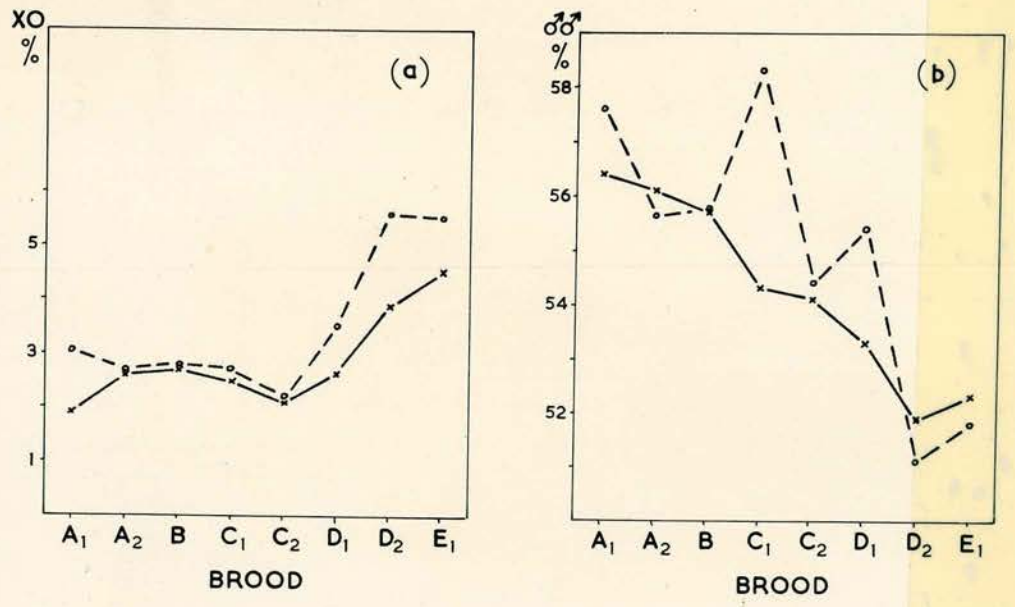
Table 2:5 The frequencies of normal males, XO males, XXY females, and gynandromorphs in experiments XIII (N_2.2kR.O_2), and XIV (N_2.2kR.N_2). Broods A, C, D and E were split and only the first day of brood E_1 was scored.

Brood	Expt.	♂♂	♀♀	n.	♂/n%	XO♂	%	XXY	%	Gyn.	%
A_1	XIII	1768	1307	3135	56.4	60	1.91	1	0.03	15	0.48
	XIV	1783	1216	3093	57.6	94	3.04	3	0.10	8	0.26
A_2	XIII	2007	1479	3578	56.1	92	2.57	4	0.11	4	0.11
	XIV	1506	1128	2707	55.6	73	2.70	1	0.04	7	0.26
B	XIII	2368	1766	4249	55.7	115	2.71	2	0.05	5	0.11
	XIV	2609	1945	4684	55.7	130	2.78	2	0.04	8	0.17
C_1	XIII	1191	946	2192	54.3	55	2.51	2	0.09	9	0.41
	XIV	1172	784	2011	58.3	55	2.73	0	0	5	0.25
C_2	XIII	927	752	1715	54.1	36	2.10	2	0.12	0	0
	XIV	1252	1000	2303	54.4	51	2.21	1	0.04	4	0.16
D_1	XIII	687	567	1288	53.3	34	2.64	1	0.08	1	0.08
	XIV	797	590	1438	55.4	51	3.55	1	0.07	1	0.07
D_2	XIII	679	578	1308	51.9	51	3.90	1	0.08	2	0.16
	XIV	673	571	1318	51.1	74	5.61	5	0.38	2	0.15
E_1	XIII	299	247	572	52.3	26	4.55	2	0.35	1	0.17
	XIV	420	346	811	51.8	45	5.55	2	0.25	1	0.13

Figure 2:3. The brood patterns for (a) XO male frequency and (b) normal male frequency in experiments XIII (x———x), N_2.2kR.O_2, and XIV (o— — —o), N_2.2kR.N_2.

Fig. 2:3 (b) shows clearly the linear decrease of normal male frequency in experiment XIII and the step-wise decrease in experiment XIV.

Fig. 2 : 3



Results: 2d, experiments XV, XVI and XVII

The dose-rate effect

In all of the foregoing experiments the X^{C2}, y B/sc^{8}•Y males were irradiated with the ENRAF machine, at a rate of 2,600R/min. It was known that the X-ray machine in the Institute of Animal Genetics, in Edinburgh, was capable of providing a dose rate of 400R/min., but probably not much faster. Therefore, two experiments were carried out with the ENRAF machine, in which 2kR was delivered at either 400R/min. (experiment XV) or 2,600R/min. (experiment XVI); nitrogen pre- and post-treatments were also used.

The data obtained in these two experiments are given in table 2:6. In both experiments (fig. 2:4a) the frequencies of XO males followed the expected brood pattern, with a marked peak in broods D_1 and D_2. There was a tendency for the frequencies of XO males to be relatively higher, in broods C and D_1, after irradiation at the low dose rate. However, this could have been caused by a small change in the rates of sperm utilization.

In experiment XVI the frequencies of normal males (2:4b) followed the expected brood pattern, with a sharp drop from brood C to broods D_1 and D_2. However, in experiment XIII the frequencies of normal males remained high through broods A, B, C, D_1 and D_2. This was the first time that such a normal male brood pattern had been found and, although time was short, it was decided to repeat the low dose-rate treatment.

The protocols of experiment XVII were identical to those of experiment XV with the one exception that brood E was split into two one-day broods. The data obtained in experiment XVII are given in table 2:7. In this table, it can be seen that the frequencies of XO males followed the expected brood pattern, while the frequencies of normal males did not show a marked drop from brood C to brood D_1. The numbers of progeny in brood D_2 were too low

to allow a realistic comparison with the other broods. The splitting of brood E showed that the XO male peak did not continue into the sperm sampled on the ninth day of sampling.

Conclusion

The change of dose rate caused a specific modification of the brood pattern of the frequencies of normal males. This modification was restricted to the broods which contained the highest frequencies of XO males and the lowest numbers of progeny and presumably represented irradiated spermatocytes. The simplest interpretation of this phenomenon was that there was a dose rate dependent mechanism, in spermatocytes, which caused non-random segregation of the sex chromosomes.

These were the final experiments in which adult males were irradiated. It had been shown that the frequencies of XO males followed a consistent brood pattern and were only slightly affected by any of the experimental procedure changes. On the other hand, the frequencies of normal males were not so consistent, being modified by each procedural change and even showing pattern variations between replica 2kR high dose rate, nitrogen post-treatment experiments. These variations occurred mainly in sperm sampled during the first six days after irradiation. On the seventh and eighth days a dose-rate dependent modification of the frequencies of normal males was found.

Throughout these experiments, other classes of progeny were also scored, but to assess the extent to which these were affected by radiation it was necessary to pool either the data from different experiments or from all the broods within each experiment. This will form the subject of the next section.

Table 2:6 The frequencies of normal males, XO males, XXY females, and gynandromorphs in experiments XV (400R/min.) and XVI (2,600R/min.). In both experiments a dose of 2kR was given with nitrogen pre- and post-treatments.

Brood	Expt.	♂♂	♀♀	n.	♂/n%	XO♂	%	XXY	%	Gyn.	%
A	XV	2839	2044	5045	56.3	162	3.21	4	0.08	27	0.53
	XVI	2464	1624	4211	58.5	123	2.92	0	0	9	0.21
B	XV	2610	1801	4541	57.5	130	2.86	2	0.04	14	0.31
	XVI	2973	2113	5223	56.9	137	2.62	3	0.06	12	0.23
C	XV	1768	1181	3067	57.6	118	3.85	4	0.13	11	0.36
	XVI	1390	999	2458	56.6	69	2.81	1	0.04	7	0.28
D_1	XV	353	215	619	57.0	51	8.24	2	0.32	2	0.32
	XVI	425	334	799	53.2	40	5.01	0	0	3	0.38
D_2	XV	205	124	360	56.9	31	8.61	2	0.56	0	0
	XVI	169	144	349	48.4	36	10.32	1	0.29	0	0
E	XV	896	751	1670	53.7	23	1.38	4	0.02	3	0.17
	XVI	695	509	1234	56.3	30	2.43	1	0.01	1	0.08

Figure 2:4 The brood patterns for (a) XO male frequency and (b) normal male frequency in experiments XV (×———×), 400R/min., and XVI (o— — —o), 2,600R/min.

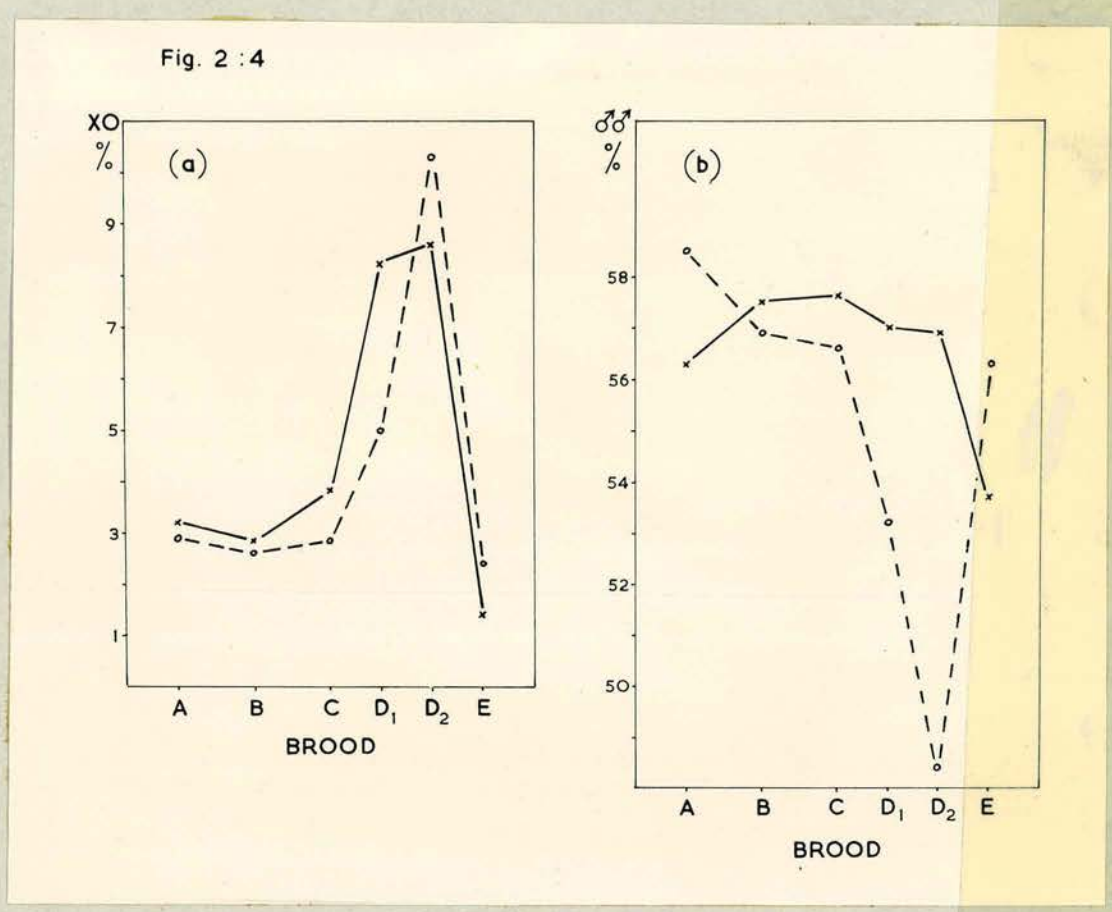


Table 2:7 The frequencies of normal males, XO males, XXY females and gynandromorphs in experiment XVII (400R/min.).

Brood	♂♂	♀♀	n.	♂/n%	XO♂	%	XXY	%	Gyn.	%
A	2023	1284	3394	59.6	87	2.56	1	0.03	8	0.24
B	1837	1152	3087	59.5	98	3.17	7	0.23	5	0.16
C	1816	1135	3051	59.5	100	3.28	2	0.06	1	0.03
D_1	350	205	600	58.3	45	7.5	3	0.50	1	0.16
D_2	36	25	67	53.7	6	8.96	1	1.49	0	0
E_1	589	441	1044	56.4	14	1.34	0	0	2	0.19
E_2	377	299	685	55.0	9	1.31	3	0.44	10	1.46

Results: 3

Analysis of the exceptional classes of progeny

The main purpose of this analysis was to find out whether the data contained any indication that a major part of the XO male peak was caused by mechanisms other than chromosome breakage and loss.

Induced crossing-over and non-disjunction might have caused the production of XO males in the spermatocyte brood. Single cross-overs, between the ring-X and the sc^{8}•Y chromosomes, would have produced dicentrics. These dicentrics could then either have caused the death of the dividing spermatocyte or caused the loss of both the X and the Y chromosome. In the first case, no XO males and no change in the frequency of normal males would have been produced. In the second case, the Y chromosome would have been lost at the same rate as the X chromosome and this would have caused a large decrease in the frequency of normal males. At the start of the investigation it had been thought that the contribution of crossing-over to the XO male peak could be estimated from the relative rates of increase of the frequencies of XO males and normal males. However, this analysis was made impossible by the finding that the frequencies of normal males were dose rate dependent and it was not possible to estimate the amount of induced crossing over.

The contribution made by non-disjunction, to the XO male peak, was estimated by comparing the numbers of phenotypically XXY females to the corresponding numbers of XO males.

A proportion of the XO males would have been caused by partial loss of the Y chromosome. Some of these losses would have left the Y chromosome with all of its fertility factors and the proportion of such losses was indicated by the number of XO males which were fertile.

Mosaic losses of the ring-X chromosome would have caused the production of gynandromorphs. It was expected that the frequencies of gynandromorphs would have been dose dependent if complete losses of the X chromosome had been caused by the formation of dicentrics and interlocking rings, and consequently bridge-breakage-fusion cycles.

Finally, there were exceptions caused by maternal non-disjunction and these were not expected to be affected by the radiation treatments.

For an analysis of the frequencies of gynandromorphs, data were pooled from all broods within each experiment. To analyse the other classes of exceptional progeny, data were pooled from the corresponging broods in experiments IX, XII, XV, XVI and XVII. These five experiments were chosen because of the similarity between the treatments and the brood patterns which were used in them. The only difference was that a high dose rate was used in experiments IX, XII and XVI while a low dose rate was used in experiments XV and XVII. The pooling of data was necessary because of the small number of exceptions in each experiment.

3a: Paternal non-disjunction

The within brood totals of XXY females, together with the corresponding XO male totals and the XXY/XO ratios are shown in the table 3:1.

Table 3:1

Brood	XXY	XO	XXY/XO
A	12	642	0.019
B	16	550	0.029
C	12	464	0.026
D_1	13	262	0.050
D_2	5	113	0.044
E	11	175	0.063

In the above table, it can be seen that the XXY/XO ratios fell into two

groups, about 0.025 in broods A, B and C, and about 0.050 in broods D_1, D_2 and E. The rise from brood C to brood D_1 was expected, on the assumption that this point in the brood sequence corresponded approximately to the change from germ cell stages which had been post-meiotic at the time of irradiation to germ cell stages which had been meiotic and pre-meiotic at the time of irradiation.

An estimation of the contribution of non-disjunction to the production of XO males is complicated by two factors. Firstly, following spontaneous paternal non-disjunction there is a tendency for more nullo-X sperm than XY-sperm to produce viable progeny, the ratio of F_1 XXY females to XO males being dependent upon the genotype of the paternal male (Spieler, 1963). Secondly, there were two ways other than paternal non-disjunction, by which phenotypically XXY females could have been produced. Transfer of the y^+ marker from the sc^8•Y chromosome to the X^{C2} chromosome by double crossing over, or transfer of the y^+ marker to an autosome as a translocation. All three mechanisms of XXY female production would have been expected to be induced by radiation in meiotic and pre-meiotic germ cells. Testing of the exceptional females was not possible because they occurred at very low frequencies and were always found to have been fertilized by males in the same culture, which carried the sc^8•Y chromosome. However, the low XXY/XO ratios indicated that non-disjunction could only have been responsible for a small proportion of the XO males, even in meiotic germ cell stages.

3b. Gynandromorphs:

It has been argued by Bonnier et al. (1949) that, if the mechanisms of chromosome breakage and loss proposed by Muller (1940) and Pontecorvo (1942) were operative, it would be expected that the irradiation of Drosophila males would lead to an increase in the frequency of gynandromorphs in the next generation. Following the irradiation of rod-X males (Bonnier and Lüning, 1951) an increase in the frequencies of gynandromorphs was indeed found.

Using a similar reasoning to that employed by Bonnier et al. (1949), it can be argued that at least one of the configurations formed by the rejoining of the ends of a broken ring-X chromsome would increase the probability of gynandromorph production. Three distinct types of dicentric chromosome formation can be formed by the breakage of a ring-X. When sister-chromatid reunion occurs then a large dicentric ring will be formed, with the distance between the centromeres dependent upon the position of the original breakage point. When the chromatids of the broken ring-X rotate through 180°, before reunion, a large dicentric ring will be formed with the centromeres equidistant, at opposite ends of the ring. Movement of the centromeres, of these two types of dicentric, to the poles of a mitotic spindle will cause the breakage of two chromatids and if this does not start a bridge-breakage-fusion cycle, then it is probable that the positions of the breakage points will lead to the formation of unbalanced chromosomes and thus to lethality.

The third type of dicentric configuration will be formed when the chromatids of a broken ring-X rotate through 360° before reunion. When the centromeres split, a pair of interlocking rings will be formed and if one of these rings breaks, then it may either be lost or cause lethality. The unbroken ring will retain the stable configuration and, when the broken ring is lost, a gynandromorph may be produced.

In the present experiments, the scoring of gynandromorphs was limited by the absence of a distinctive recessive marker on the maternal X chromosome. Both the paternal and maternal X chromosomes carried the recessive marker yellow (y).

However it was possible to score all those cases in which the areas of XO tissues included sexually dimorphic regions of the body and also those cases in which the sides of the head were either XO or XX. The identification of gynandromorphs was also aided by the phenotype of y sc^{S1} In49 sc^{8}/O tissue, which carried fewer and smaller bristles than either XX or XY tissue.

In some experiments (VII, VIII, IX, XI, XII, XIII, XV and XVII) there was a tendency for the frequencies of gynandromorphs to be higher in brood A than in the subsequent broods. Rather than being an effect of radiation, this was probably a confirmation of the observation made by Brown and Hannah (1952), that the frequencies of gynandromorphs decreased with the increasing age of the paternal male. To find out whether there had been a dose-effect on the frequencies of gynandromorphs, the data from all the broods within each experiment were pooled. The total data are given in the table below.

Table 3:2

Experiment	Dose (kR)	Gynandromorphs	n.	Percentage of gynandromorphs
VI	0	67	37,079	0.18
V	0.5	59	27,430	0.22
III	1	8	15,318	0.05
IV	1	72	28,314	0.25
II	2	13	25,467	0.05
IX	2	15	15,578	0.10
X	2	25	18,228	0.14
XI	2	37	21,271	0.17
XII	2	31	19,684	0.16
XIII	2	37	18,037	0.21
XIV	2	36	18,365	0.20
XV	2	57	15,302	0.37
XVI	2	32	14,274	0.22
XVII	2	27	11,928	0.23
I	3	25	20,081	0.12
VII	3	21	13,453	0.16
VIII	3	11	11,821	0.09

In table 3:2 it can be seen that there was no tendency for the frequencies of gynandromorphs to be dose dependent. Therefore it was concluded that the radiation sensitive mechanism which caused the high frequency of complete losses of the ring-X chromosome did not cause the production of gynandromorphs. The spontaneous origin of the gynandromorphs was further indicated by the fact that the frequencies given in the last column of

table 3:2 are in the range expected when unirradiated ring-X males are mated to young females (Brown and Hannah, 1952).

Together with gynandromorphs, XX:XO mosaics, there was another class of progeny which represented the mosaic loss of an entire chromosome. These were XY:XO mosaics, of which only four were found during the entire period of investigation. These exceptional males were all bilateral half and half mosaics and all four of them were sterile.

Thus there was no evidence to indicate that the frequencies of either XX:XO or XY:XO mosaics were affected by the irradiation of the paternal males.

3c: XO males

In the early experiments a few XO males were tested for fertility and it was found that only a very small proportion of them were fertile. A quantitative analysis was made with XO males from experiments XV and XVI. The XO males were individually mated to pairs of females from the maternal, homozygous y stock and the matings were scored for fertility after eight days. The cultures which contained larvae were allowed to develop and were then scored for the segregation of y and y^+. The data obtained from these fertility tests are given below in table 3:3.

Table 3:3

Experiment				XV	XVI
XO males tested				371	429
Sterile				365	427
Fertile (F_2)					
♂♂		♀♀			
y	y^+	y	y^+		
+	—	+	—	4	1
—	+	+	—	2	0
+	+	+	—	0	1

The proportion of fertile XO males (8/800) was very low and consistent with the proportion of fertile XO males (2/150) which Sävhagen (1963) found in the progeny of irradiated rod-X/sc^{8}·Y males. Using the singly marked Y chromosome it was not possible to distinguish between loss of only the marker and loss of the entire Y or X chromosomes. Thus the 1% fertility was based on the total of all partial and complete losses.

This difficulty could have been avoided by the use of a doubly marked Y chromsome, such as $B^{S}Yy^{+}$ which was derived from the sc^{8}·Y chromosome. However, it was suspected that the different structures of the two Y chromosomes might cause them to have inherently different sensitivities. This was confirmed when the data given above were compared to the frequencies of fertile 'complete' and partial losses which other authors obtained after the irradiation of males with a $B^{S}Yy^{+}$ chromosome. Fahmy and Fahmy (1964), Snyder and Oster (1964) and Zimmering and Kirshenbaum (1964) all found that about a third of the single Y chromosome marker losses were fertile. In addition, Fahmy and Fahmy found that 7% of the 'complete' losses were fertile, while Snyder and Oster found that 1% of the 'complete' losses were fertile. Thus, when the numbers of 'complete' and y^{+} losses are summed and the proportion of fertile males is calculated, this is found to be considerably in excess of the 1 - 1.5% fertility typical of the XO males in the progeny of irradiated sc^{8}·Y males. It seems probable that the different structures of the sc^{8}·Y and the $B^{S}Yy^{+}$ chromosomes are responsible for this discrepancy. The x y^{+} marker is located on the long arm of the sc^{8}·Y chromosome and on the short arm of the $B^{S}Yy^{+}$ chromosome. It is also possible that the marker is more closely associated with a fertility factor on the sc^{8}·Y chromosome.

While the proportions of fertile losses appeared to be dependent upon the irradiated Y chromsome genotype, the proportion of fertile males which

were mosaic appeared to be independent of the type of Y chromosome. Thus in experiments XV and XVI, 3 out of 8 fertile males were mosaic and Fahmy and Fahmy also found that slightly less than a half of their fertile exceptions were mosaic. These mosaics could be gonad:soma mosaics or part-gonad soma: part-gonad mosaics, but more complicated configurations were also possible. 305 XO males, from experiments of Sobels and Tates (personal communication) were tested by the author. Six of these males (2%) were fertile, which is consistent with the proportion expected in the progeny of sc^8·Y males. One of these males produced an anomalous F_2 segregation. There were 45 y^+ ♂♂, 21 y ♀♀ and 23 y^+ ♀♀, and on further testing it was found that the y^+ females and half of the males carried the y^+ marker on chromosome IV. This mosaic appeared in the progeny of irradiated mature sperm and probably arose as a Y-IV chromatid exchange occurring before the first mitotic division. When the chromosomes segregated the proto-gonad nucleus received two y^+ markers, one on the Y and the other on chromosome IV, while the proto-hypoderm nucleus received no y^+ marker.

Since many of the partial losses of the Y chromosome must have been sterile and probably a relatively large proportion of the mosaic losses would have appeared to be normal males, a direct estimate of the contribution of losses of the Y chromsome to the production of XO males was not possible. No quantitative testing of the fertility of normal males has yet been carried out but it was noted, in the sex-linked lethal tests, that a number of the F_2 cultures were sterile while in some of the fertile ones there was a segregation of y and y^+ in the F_2 generation. The main evidence that losses and partial losses of the Y chromosome make only a minor contribution to the production of XO males comes from the data of Baker (1957) who irradiated rod-X and ring-X males which carried the same bw^+Y y^+ chromosome. In mature

sperm he found that the frequencies of induced partial losses of the Y chromosome were independent of the type of X chromosome. That this is probably the case in all germ cell stages is indicated by the fact that the frequencies of XO males induced in the spermatocyte brood of irradiated rod-X/sc^8·Y males (Savhagen 1961) were less than a tenth of those found after the comparable irradiation of the spermatocytes of ring-X/sc^8·Y males, in the present experiments.

3d: Maternal non-disjunction

Two types of viable exceptions were expected as a consequence of maternal non-disjunction, XX/Y and XX/O females. These females would have been round-eyed and either y^+ or y. 47 round-eyed females were found in experiments IX, XII, XV, XVI and XVII, in a total of 76,766 F_1 progeny. This gave a rate of maternal non-disjunction of 1 in 1,633, which is very close to the rate of 1 in 1,735 that was found by Merriam and Frost (1964). Only four of the round-eyed females were yellow, which is a relatively high proportion (4/47 = 0.085) and it is possible that these females were gynandromorphs in which the heads were XO and the thoraxes and abdomens were XX.

3e: Conclusion

An analysis of the exceptional classes of progeny did not provide any indication that causes other than induced ring-X chromsome loss were responsible for more than a small proportion of the XO males.

Results: 4

The irradiation of pupae

Pupae were irradiated, to obtain a more direct sampling of the germ cell stages which were most sensitive to the induction of X^{C2} chromosome loss. Khishin (1954) has shown that spermatids are the most advanced germ cell stage in the developing testis of 48 hour pupae. Therefore, 48 hour pupae were irradiated and it was intended to take several broods from the emergent males. However this was found to be impracticable because the irradiation of pupae in nitrogen caused a slowing-down of development, a spread of hatching time, and an increased pre-hatching mortality. These three effects are shown in table 4:1, below.

Table 4:1

Experiment	XVIII	XIX	XX
Dose (N_2 pre- and post-)	1.5kR	1.0kR	0.5kR
Pupae irradiated	80	80	80
Hatched			
96 - 120 hr.	42	49	61
120 - 150 hr.	13	17	14
Total	55	66	75
Percentage mortality	31.3	17.5	6.2

In the bottom row of table 4:1 it can be seen that the induced pre-hatching mortality was dose dependent. This indicated that it was caused by the irradiation. However, neither pre-hatching mortality nor developmental delay was induced in an irradiation-only control experiment. Similarly, a nitrogen-only control experiment had no effect on the developmental time or the proportion of pupae which hatched.

Thus, a particular kind of damage was induced only when pupae were irradiated in a nitrogen atmosphere. It should be noted that Falk (1962) reported that extended nitrogen treatments caused a developmental delay, but this was not dependent on the radiation treatment which was given separately.

Further experiments with pupae might have provided a basis for understanding the nitrogen effect (Thoday and Read,1949) but this was outside the scope of the present investigation and therefore no further experiments have been carried out with pupae.

DISCUSSION

The original aim of this investigation was to find out whether chromosome breakage was the main cause of the XO male peak which occurred in the spermatocyte brood of irradiated Drosophila melanogaster males. Non-disjunction and crossing-over were two possible alternative causes of the XO male peak. In experiments with rod-X males (Sävhagen 1961, Strangio 1962) the XO male peaks were not high enough to demonstrate convincingly that chromosome breakage was indeed the main cause. The available evidence (Pontecorvo 1940, Lüning 1952, Baker 1957) indicated that ring-X chromosomes were lost at a far higher rate than rod-X chromosomes. Therefore it appeared that a study on the effects of radiation on ring-X males would permit a finer resolution of the contributions made by chromosome breakage, non-disjunction, and crossing-over to the XO male peak.

Previous authors have proposed that breakage of the ring-X chromosome causes chromosome loss and lethality. This hypothesis provided an explanation for the observation that the progeny of irradiated ring-X males contained high frequencies of XO males and large deficiencies of females. A correlation was found between these two effects of radiation and it was argued that they were caused by one type of primary event, namely breakage of the ring-X chromosome. The data obtained in the first experiments of the present study indicated that the assumption of a correlation between the frequencies of XO males and the increases in the frequencies of normal males was possibly an oversimplification. This tentative conclusion was supported by the data obtained in later experiments. It will be proposed that the increases in the frequencies of XO males and the increases in the frequencies of normal males are measures of two different kinds of damage. The primary cause of the XO males has not been determined, but it

is possible to explain the increases in the frequencies of normal males as being caused by a kind of damage which is reparable.

The conclusion that the cause of the XO males is different from the cause of the increases in the frequencies of normal males was based on an accumulation of evidence. Firstly, there was the contrast between the uniformity of the XO male dose response curves in broods A, B and C and the irregularity of the normal male dose response curves in these three broods. Then, in a series of experiments, with a dose of 2kR, it was found that the frequencies of XO males followed a regular brood pattern and there was good agreement between the frequencies of XO males in the corresponding broods of different experiments. No significant modification of the induced frequencies of XO males was caused by such treatment changes as post-treatment with oxygen, removal of "O_2-multirapid" from the nitrogen, or a change of the radiation dose rate. In marked contrast, the changes in the frequencies of normal males were irregular. Inconsistencies were found between the corresponding broods in replica experiments. Superimposed on this basic instability, evidence was obtained which indicated that significant modifications of the frequencies of normal males were caused by such treatment changes as oxygen post-treatment, removal of "O_2-multirapid" from the nitrogen, and the use of different brood intervals. In addition, it was found that the frequencies of normal males were dose rate dependent in the spermatocyte brood, while the corresponding frequencies of XO males were only slightly affected.

Thus the XO males appeared to be caused by a stable kind of damage and the changes in the frequencies of normal males appeared to be caused by an unstable kind of damage.

Following the irradiation of ring-X males, inconsistent sex ratio shifts have been reported by Bender (1958) and Lindsley et al. (1963). Therefore this

puzzling response to radiation may be considered as a general property of ring-X males, although stable responses have been reported by Bauer (1939) and Baker and von Halle (1955). This variation in response is paralleled by the degrees of instability of the w^{vc} chromosome and its rod derivatives (Hinton 1955, 1957). Hinton suggested that the spontaneous instability might be under genetic control and it is possible that there is a genetic modifying system which affects the response of stable ring-X chromosome males to irradiation.

The frequencies of XO males, whatever their primary cause, showed a very regular response to radiation. If this regularity of response is taken as evidence for XO males being a rather direct consequence of radiation, then it follows that the irregularity of the frequencies of normal males was caused by secondary modifying processes. There are two reasons for assuming that the irregularity was particularly associated with ring-X bearing sperm and zygotes formed by such sperm. Firstly, it has been argued that the Y chromosome makes very little contribution to the letality of zygotes formed by Y-bearing sperm (Lindsley in discussion after Lindsley et al. 1963) and secondly, irregularities of sex ratio are not found in the progeny of irradiated rod-X males (for example Sankaranarayanan 1964, Yanders 1965). Presumably the irregularities are caused by an instability of the processes of repair and fixation of potentially lethal lesions induced in ring-X bearing germ cells.

The alternative explanation of the data is that the increases in the frequencies of normal males were a direct measure of induced damage and the frequencies of XO males were secondarily modified. This interpretation is considered improbable for two basic reasons. It is unlikely that the induction of primary damage was irregular and that secondary modification

produced the regularity of the XO males in a large number of experiments. Secondly, in the post-meiotic broods there were significant differences between the frequencies of normal males within experiments as well as between experiments and this was not so for the frequencies of XO males.

It has generally been assumed that when rod-X males are irradiated there is only a slight increase in the proportion of male progeny. This is indeed the case when the irradiated rod-X males are mated to free-X females. However large sex ratio shifts have been found when irradiated rod-X males were mated to attached-X females. Barth (1929) irradiated rod-X males and mated them to free-X females and attached-X females. In the first cross the X-bearing sperm produced female progeny and in the latter cross the X-bearing sperm produced male progeny. Barth's data are difficult to interpret because he carried out no control experiments and assumed expected frequencies of 50% male progeny from both types of cross. In addition, he measured the induced shifts as the ratio of the deviation from 50% over the "standard error". The latter factor was dependent on the number of progeny scored and the number of progeny from the crosses to free-X females was several times larger than the number of progeny from the crosses to attached-X females. Nevertheless, there was a greater sex ratio shift in the progeny of the attached-X females. These experiments were repeated by Abrahamson (1961) who confirmed Barth's result and in addition found that the deficiency of male progeny, in the attached-X cross, was several times greater than could be accounted for by the frequencies of recessive sex-linked lethals in the daughters of the free-X cross. Abrahamson proposed that one possible explanation of this discrepancy was that X-bearing egg pronuclei were able to cause the repair of a greater amount of potentially lethal damage, in sperm nuclei, than Y-bearing egg pronuclei. The

observation that the deficiency of male progeny, in the attached-X cross, was several times greater than could be accounted for by the induced frequencies of recessive sex-linked lethals, indicated that the repair of potentially lethal damage in XX zygotes could be complete.

A third sex ratio phenomenon has been reported by Lindsley et al. (1963). They irradiated rod-X males which carried various modified rod-X chromosomes and found that the sex ratio shift in the progeny of these males was dependent on the structure and not the length of the paternal X chromosome. In particular, a large amount of heterochromatin at the distal end of the X chromosome caused an increased sensitivity to X-ray induced sex ratio shift.

The data from the present investigation, the data of Barth and Abrahamson, and the data of Lindsley et al. cannot be explained by the chromosome breakage hypothesis. From this hypothesis it would have been predicted that there would be a correlation between the frequencies of XO males and the increases in the frequencies of normal males in the progeny of irradiated ring-X males. Secondly, the lethal consequences of a break in a rod-X chromosome should not have been affected by whether the female pronucleus contained an X chromosome or whether it contained a Y chromosome. Thirdly, the amount of induced chromosome breakage should have been more dependent on the length of the paternal X chromosome than on the structure of the paternal X chromosome.

On the other hand, all of the data can be explained if it is assumed that lethality is caused by repairable damage and the amount of repair is dependent on an unknown relationship between the paternal and maternal sex chromosomes. In the examples given above there were marked structural differences between the maternal and paternal sex chromosomes. It might, however, be expected that the amount of repair would be affected by differences which were less marked.

This would account for the discrepancies between the results of very similar experiments carried out by different investigators. These discrepancies have been reported in the early literature (Demerec and Fano 1944, Lea and Catcheside 1945), and there are also very recent examples. Males carrying normal rod-X chromosomes have been irradiated and mated to free-X females by Moriwaki et al. (1961), Lindsley et al. (1963), Sankaranarayanan (1964) and Yanders (1965). Moriwaki et al. and Yanders reported that radiation did induce sex ratio shifts, while Lindsley et al. and Sankaranarayanan found no induced sex ratio shifts. It cannot be argued that the negative results of both the last two authors were caused by the use of too low a radiation dose, because Sankaranarayanan irradiated the males in each generation, for 25 generations, with doses as high as 6kR.

Radiation induced sex ratio shift in Drosophila is a complex phenomenon. To explain all of the data it is necessary to find a hypothesis which will predict inconsistencies. This requirement is fulfilled by the assumption of reparable lesions whose induction and repair is dependent on the paternal and maternal genotypes. It can be further argued that repair, when it occurs, is complete. Thus the induced lesions are either eliminated by the lethality which they cause or they are completely repaired. In neither case is there a transmission of genetic damage.

Non-transmissible genetic damage, because of its inherent properties, is difficult to detect and recognise. It has, however, been found in Neurospora, yeast, and Habrobracon.

Norman (1951a) made a comparative study of the UV inactivation of Neurospora microconidia and macroconidia, using the kinetic model developed by Atwood and Norman (1949). He found that the individual nuclei in multinucleate

macroconidia were intrinsically more radioresistant than the individual nuclei in microconidia. Atwood (1950) had shown that recessive lethal mutations were not an important cause of the UV inactivation of macroconidia. Therefore Norman argued that there were two types of damage which caused the inactivation of Neurospora conidia, transmissible damage and non-transmissible damage. Non-transmissible damage was able to cause the lethality of microconidia but could be repaired by undamaged nuclei in macroconidia. The dose response curves indicated that there was a relationship between the two types of damage. This was further indicated by the fact that both types of damage were reversed to about the same extent by photoreactivation (Norman 1951b).

The assumption of a relationship between transmissible damage and non-transmissible damage was supported by the results from later experiments of Atwood (1954). Multinucleate conidia from a heterokaryon were irradiated and the decrease in the proportion of heterokaryotic cells was estimated by comparing the survival rates on minimal and supplemented medium. A reduction in the proportion of heterokaryotic cells, with increasing dose, was expected as the proportion of undamaged nuclei was reduced. The expected reduction in the proportion of heterokaryotic cells was found when X-irradiation was used. When UV was given there was very little reduction in the proportion of heterokaryotic cells, even at very low survival rates. However, when the reduction in the proportion of heterokaryotic cells was estimated by plating the irradiated conidia on supplemented medium and then testing the ability of the developing colonies to grow on minimal medium, it was found that UV did induce a reduction in the proportion of heterokaryotic cells. Atwood (1954) explained this discrepancy by assuming that undamaged nuclei were able to repair UV induced damaged in other nuclei, in the same cell, when the initial divisions were retarded by minimal medium.

The X-ray inactivation of haploid and diploid yeast was studied by Latarjet and Ephrussi (1949). The survival curves which they obtained were redrawn by Norman (1951a) who found that these were very similar to the UV survival curves of Neurospora microconidia and macroconidia. Therefore Norman suggested that the inactivation of yeast cells was caused by transmissible damage and non-transmissible damage. Magni (1956) confirmed that diploid yeast cells were considerably more radioresistant than haploid yeast cells. He was also able to show that this difference in sensitivity could be only partially accounted for by the frequencies of recessive lethals which were induced in the diploid yeast cells. There are two other lines of evidence which indicate that non-transmissible genetic damage is induced in yeast. Firstly, Beam (1959) found that the radioresistance of haploid yeast was greatly increased during a particular period of the budding process and the frequencies of recessive lethals induced in the resistant cells were not high enough to account for the decreased sensitivity. Secondly, Mortimer (1955) irradiated haploid yeast cells and then mated them to unirradiated haploid cells. Although the doses of radiation were high enough to produce an expected inactivation of over 99% of the haploid cells, the mating enabled a very high frequency of survival. Mortimer (1955) noted that the mating of irradiated haploid cells to unirradiated haploid cells was followed by a considerable delay of the first and second zygotic divisions. This delay was typical of the effect produced when diploid yeast cells were irradiated (Holweck and Lacassagne 1930). Magni (1959) and Mortimer (1961) repeated the mating experiments and confirmed Mortimer's results. Magni tested the viable zygotes for recessive lethals and found that the observed frequency did not account for the difference in survival between the mated and the unmated haploid yeast.

von Borstel (1961) found that the fertilization of Habrobracon oocytes which had been treated with X-rays caused a small but consistent reduction in the amount of expressed type I dominant lethality. Following the irradiation of Habrobracon eggs with low doses of UV, there was a higher survival of the fertilized eggs than of the unfertilized eggs. This phenomenon was investigated in detail by Löbbecke and von Borstel (1963). Freshly laid Habrobracon eggs were irradiated with UV, before the male and female pronuclei had fused. At low doses the fertilized eggs were more resistant than unfertilized eggs and this difference was found to be independent of photoreactivation. In Habrobracon, fertilized eggs produce females and unfertilized eggs produce males. Therefore it was possible to compare the sex ratio in the progeny of eggs from mated females with the differential survival rates. The proportion of females in the total progeny increased with increasing dose and this indicated that there was a preferential survival of fertilized eggs. The F_1 females were tested for recessive lethals and it was found that the induced frequencies of recessive lethals were too low to account for the increased survival rates of fertilized eggs. The increased survival of fertilized eggs was accompanied by a considerable reduction in the proportion of type I embryo lethality. The type I lethality syndrome (von Borstel 1959) is caused by a slowing down of the first nuclear divisions, followed by a complete cecession of mitosis and a swelling of the nuclei which have been produced. Thus nuclear reactivation in Habrobracon operates under conditions which would normally cause a retardation in the rate of nuclear division.

In three organisms, Neurospora, yeast, and Habrobracon, identical phenomena have been described. Undamaged haploid nuclei are able to cause the repair of

a considerable amount of damage which has been induced in other haploid nuclei. In all three organisms the repair mechanism operates during a period of retarded nuclear division.

One discrepancy would appear to be that different types of radiation cause different amounts of reparable damage in the three organisms. UV induces a large amount of reparable damage in Neurospora and Habrobracon and X-rays induce a large amount of reparable damage in yeast but only a small amount of reparable damage in Habrobracon and no detectable amount of reparable damage in Neurospora. This discrepancy may be caused by unknown differences between the actions of X-rays and UV on biological material.

In Drosophila the irradiation of haploid post-meiotic germ cells is analogous to the irradiation of Neurospora microconidia, haploid yeast cells, or unfertilized Habrobracon eggs. It is, however, not possible to test the viability of haploid sperm. Fertilization of the egg is analogous to producing the conditions within a Neurospora macroconidium, a yeast zygote, or a fertilized Habrobracon egg and most of the non-transmissible damage will normally be repaired. When Barth (1929) and Abrahamson (1961) mated irradiated rod-X males to attached-X females, the X-bearing sperm was expected to form viable progeny by fusion with a Y-bearing female pronucleus. Large deficiencies of male progeny were found and these could be only partially accounted for by the induced frequency of recessive sex-linked lethals (Abrahamson 1961). This is a typical indication of the induction of non-transmissible genetic damage. Thus in Drosophila the repair of non-transmissible genetic damage is governed at the chromosomal level and is dependent on the degree of homology between the chromosomes in the irradiated and the unirradiated haploid gametic nuclei. From this hypothesis much of the apparently anomalous X-ray induced sex ratio shift data can be

explained. The different sex ratio shifts found in the progeny of irradiated normal rod-X males mated to free-X females will be caused by minor differences between the irradiated rod-X chromosomes and the X chromosomes of the female tester stocks. These differences may be magnified when the irradiated X chromosomes carry large inversions or duplications and therefore Lindsley et al. (1963) found significant sex ratio shift in the progeny of irradiated $In(1)sc^{8}$ males and $In(1)sc^{S1L},sc^{4R}$ males. Ring-X chromosomes differ in structure from rod-X chromosomes. Therefore when haploid ring-X bearing sperm are irradiated and fertilize rod-X bearing female pronuclei there is a high probability that the non-transmissible genetic damage will be expressed as lethality. Thus the deficiencies of females in the progeny of irradiated ring-X males can be accounted for without assuming that the lethality is a consequence of chromosome breakage.

It is not being argued that non-transmissible genetic damage is a single type of damage. The modifying effects of oxygen post-treatment and a change of the brood intervals can be most easily reconciled with the assumption of a heterogeneous class of damage. Removing "O_2-multirapid" from the nitrogen appeared to reduce the amount of non-transmissible damage and this might be analogous to the different amounts of non-transmissible damage induced in Habrobracon by X-rays and UV.

The dependence of the frequencies of normal males in the post-meiotic broods on all of the experimental protocols and the availability of a large number of modified sex chromosomes offer considerable scope for a further study on the induction of non-transmissible genetic damage in Drosophila. In addition, embryo lethals in Drosophila can be classed into several types (von Borstel and Rekemeyer 1959). Type I dominant lethality in Drosophila is

phenotypically very similar to type I dominant lethality in Habrobracon and it would be very interesting to find out whether the amount of expressed non-transmissible damage, in Drosophila, was correlated to the amount of type I dominant lethality.

The analogy between Drosophila on the one hand and Neurspora, yeast, and Habrobracon on the other, can be extended to provide an explanation of the data in the pre-meiotic broods. In these broods the frequencies of normal males were always at about the spontaneous level. The radioresistance of the diploid spermatogonia parallels the radioresistance of the Neurospora macroconidia, the diploid yeast, and the fertilized Habrobracon eggs.

It has been suggested (page 43) that the dose rate dependence of the frequencies of normal males, in the spermatocyte brood, might be caused by induced meiotic drive. Another possible interpretation of this phenomenon can now be given. The meiotic cells are a transitionary stage between the resistant gonia and the sensitive spermiogenic cells. Unknown dose rate dependent effects might influence the amount of non-transmissible genetic damage which is induced in spermatocytes.

The present study began from an observation on the induced frequencies of XO males. It was thought that breakage of the ring-X chromosome caused loss and lethality and that the induced frequencies of XO males would be correlated to the induced increases in the frequencies of normal males. The data indicated that this interpretation was an oversimplification and an alternative explanation of the induced increases in the frequencies of normal males has been given.

This leaves the problem of what was the primary cause of the ring-X chromosome loss which produced the XO males. At present this question cannot be answered, but two points can be made. Firstly, the mechanism of induced loss is related to chromosome structure (Pontecorvo 1940, Baker 1957). Secondly the mechanism which causes the complete loss of the ring-X chromosome is different from the mechanism which causes the mosaic loss of the ring-X chromosome. This is indicated by the observation that the irradiation of ring-X males did not cause an increase in the frequency of F_1 gynandromorphs.

Summary

In preliminary experiments it was found that a very high XO male peak occurred in the spermatocyte brood of irradiated ring-X Drosophila melanogaster males. The present investigation was initially designed to find out whether chromosome breakage was the main cause of this XO male peak. One-day old ring-X males were irradiated in nitrogen, with nitrogen pre- and post-treatments. A series of broods were taken from the irradiated males and the progeny were scored for increases in the frequencies of XO males and increases in the frequencies of normal males. These two effects were assumed to be measures of the amount of induced ring-X chromosome breakage.

Dose response curves were obtained for XO male frequency and normal male frequency, in the various germ cell stages. These experiments confirmed that the XO male peak occurs in the spermatocyte brood. In post-meiotic broods the dose response curves for XO male frequency were remarkably uniform and this contrasted with the irregularities of the dose response curves for normal male frequency.

For further investigation, a dose of 2kR was chosen as the optimum level. Various treatment modifications were used, such as oxygen post-treatment, removal of "O_2-multirapid" from the nitrogen, the use of different brood intervals, and a change of the dose rate. In post-meiotic broods the frequencies of XO males were repeatable and were not significantly modified by any of the treatment changes. The corresponding frequencies of normal males were more irregular and appeared to be modified by each of the treatment changes. The XO male peak occurred in the spermatocyte brood and in this

brood the frequencies of normal males were dose rate dependent. In the pre-meiotic brood the frequencies of XO males were low and the normal male frequencies were at about the spontaneous level.

It was concluded that two different kinds of radiation induced damage were being measured by on the one hand the frequencies of XO males and on the other hand the frequencies of normal males. The hypothesis is proposed that the increases in the frequencies of normal males were caused by non-transmissible genetic damage, such as has been found in Neurospora, yeast, and Habrobracon. This hypothesis provides a satisfactory explanation for several apparently anomalous results which have been reported by other authors.

The primary cause of the XO males is not known.

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ABSTRACT OF THESIS

Name of Candidate BARRY LEIGH B.Sc.

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Title of Thesis A Study on the Genetic Effects of Irradiating Drosophila melanogaster Males which carry Ring-X Chromosomes

In preliminary experiments it was found that a very high XO male peak occurred in the spermatocyte brood of irradiated ring-X Drosophila melanogaster males. The present investigation was initially designed to find out whether chromosome breakage was the main cause of this XO male peak. One-day old ring-X males were irradiated in nitrogen, with nitrogen pre- and post-treatments. A series of broods were taken from the irradiated males and the progeny were scored for increases in the frequencies of XO males and increases in the frequencies of normal males. These two effects were assumed to be measures of the amount of induced ring-X chromosome breakage.

Dose response curves were obtained for XO male frequency and normal male frequency, in the various germ cell stages. These experiments confirmed that the XO male peak occurs in the spermatocyte brood. In post-meiotic broods the dose response curves for XO male frequency were remarkably uniform and this contrasted with the irregularities of the dose response curves for normal male frequency.

For further investigation, a dose of 2kR was chosen as the optimum level. Various treatment modifications were used, such as oxygen post-treatment, removal of "O_2-multirapid" from the nitrogen, the use of different brood intervals, and a change of the dose rate. In post-meiotic broods the frequencies of XO males were repeatable and were not significantly modified by any of the treatment changes. The corresponding frequencies of normal males were more irregular and appeared to be modified by each of the treatment changes. The XO male peak occured in the spermatocyte brood and in this brood the frequencies of normal males were dose rate dependent. In the pre-meiotic brood the frequencies of XO males were low and the normal male frequencies were at about the spontaneous level.

/ It was concluded

Use other side if necessary.

It was concluded that two different kinds of radiation induced damage were being measured by on the one hand the frequencies of XO males and on the other hand the frequencies of normal males. The hypothesis is proposed that the increases in the frequencies of normal males were caused by non-transmissible genetic damage, such as has been found in Neurospora, yeast, and Habrobracon. This hypothesis provides a satisfactory explanation for several apparently anomalous results which have been reported by other authors.

The primary cause of the XO males is not known.