MUTAGENIC SENSITIVITY OF SPERM, SPERMATIDS, SPERMATOCYTES AND SPERMATOGONIA IN *DROSOPHILA MELANOGASTER*

A. C. CHANDLEY and A. J. BATEMAN

Christie Hospital and Holt Radium Institute, Manchester

1. INTRODUCTION

LÜNING (1952*a*), measuring dominant lethal incidence in successive days' matings following a single dose of X-rays in *Drosophila melanogaster*, found a variation in sensitivity in post-meiotic stages, which he confirmed by his work with hyperploids (1952*b*). This variation was attributed to hypersensitivity of the early post-meiotic stages, that is, spermatids. Auerbach (1954), using three-day " brood " periods and testing for recessive autosomal and sex-linked lethals found a peak mutational response in the second brood *i.e.* 4-7 days after irradiation (which she equated to spermatids at irradiation). This was followed by a period of high sterility in the third brood.

This hypersensitivity of spermatids has now been established in *D. melanogaster* for dominant lethals (Lüning, 1952*a*, Bateman, 1956); hyperploids (Lüning, 1952*b*, Bateman, 1957); autosomal and sex-linked lethals (Auerbach, 1954, Oster, 1955, 1956.); and translocations (Oster, 1955, 1956); and also in *D. virilis* for dominant lethals and translocations (Alexander and Stone, 1955).

In the " brood pattern " technique of Auerbach (1954) where each brood is the product of three days' matings, the mutation rates measured will represent average effects in what may often be heterogeneous material. Furthermore, the contribution of each day's mating to a brood will be weighted according to its fertility which itself will be expected to vary systematically, and thus a bias will be introduced into the overall brood result.

In the present study, it was desired to determine whether the changes in sensitivity were the same for different sectors of the mutation spectrum. For this purpose, dominant lethals, deleted X's, and translocations (all structural changes), autosomal and sex-linked lethals (mainly gene changes) were followed in the same material. At the same time evidence of induced crossing-over in the *b pr vg* region of chromosome II was obtained, primarily for its value in identifying spermatogenic stages, though it proved to have a value in its own right.

It would have been cumbersome to follow *daily* changes in all these mutations, so four days were selected as being most representative. The second day after irradiation of the male showed the minimum sensitivity of sperm to dominant lethals. The eighth day had shown

the maximum sensitivity for deleted X's and was the last day for high dominant lethal rate. The eleventh day showed a return to control values for deleted X's and dominant lethals and almost certainly represented spermatogonia surviving irradiation. The fifth day was chosen as this was half-way in time between the second and eighth.

2. MATERIALS AND METHODS

The males which were used throughout the experiment were the F_1 hybrid between Birmingham Outbred females and *b pr vg* males.

For each run, 25 F_1 males which had emerged overnight were irradiated in the morning with 1000r X-rays (a Resomax X-ray tube run at 300 kV was used for all exposures). Immediately after treatment they were mated to F_1 females at the rate of two per male per day. This mating rate was used throughout the course of the experiment and was chosen in order to ensure that sperm was used as it matured and was not stored (Bateman, 1956) a situation which would lead to the mixing of successive batches of sperm and confuse the sensitivity pattern of the several spermatogenic stages.

On day 2, which was the first sampling day, the males were divided into five equal groups A-E, the five males in each group being allocated to a different type of female in the ratio of two females to each male.

Group A males were mated to Muller-5 females for the detection of sex-linked recessive lethals.

Group B males were mated to $\frac{CyL}{Pm}, \frac{H}{Sb}$ stock females for the estimation of autosomal recessive lethals and translocations. Group C males were back-crossed to *b pr vg* females in order to detect induced crossing-over.

Group D males were mated to F_1 females and the rate of dominant lethals was scored as the percentage non-hatching eggs (using the technique of Bateman, 1956). The dominant lethal rate was used to check that each batch of males was conforming to the usual pattern in sensitivity and rate of spermatogenesis. An unirradiated control series was run concurrently.

In Group E, the males were mated to *y v f car* attached—X females for the determination of deleted X's, which were recognised in the progeny as non-yellow (*i.e.* hyperploid) daughters.

This procedure was repeated on each of the other sampling days, 5, 8 and 11. Sometimes, during the periods between the main sampling days, the males were mated to *b pr vg* females instead of F_1 females in order to follow the incidence of induced crossing-over. In this way, the daily frequency of crossing-over was obtained from day 2 up to and including day 13.

Successive runs of the experiment were made until sufficient data had been obtained for our purposes.

In estimating standard errors for some of the mutation rates we have followed the advice of Stevens (1942) in allowing for the skewness of probability distributions at low frequencies. Instead of the conventional $\pm$ symbol for the standard error we have quoted separately the upper and lower limits at the probability level of 0·15, which is the level corresponding to the standard error.

3. RESULTS

(i) *Dominant lethals*

Table 1 shows the percentages of unhatched eggs on the four sampling days for control and treated males. From this the reader can judge the consistency of the ten successive runs. The overall mean percentages are also given with their standard errors based upon

variation between runs. Fig. 1*a* shows the variation in dominant lethal rate graphically for both irradiated males and unirradiated controls, with the results of Bateman (1956) for comparison. The incidence of dominant lethals rises sharply from day 2 to day 5 and rises further to a peak on day 8. This second rise could be spurious,

TABLE 1

Percentage of non-hatching eggs following 1000r *X-rays to males*

Day		Run										Mean per cent.
		1	2	3	4	5	6	7	8	9	10	
2	Control .	—	1·76	0·33	0·93	12·30	0·23	9·93	0·82	0·89	4·90	3·56
	Irrad. .	8·01	15·34	13·60	9·50	14·10	8·19	9·05	9·52	9·14	9·40	10·59±0·85
5	Control .	2·04	0·88	0·83	0·25	1·33	0·69	0·54	0·78	0·72	0·50	0·86
	Irrad. .	48·13	29·55	41·70	31·95	27·00	59·84	53·90	43·97	34·40	40·50	41·09±3·39
8	Control .	0·80	1·89	1·40	1·00	5·90	0·00	0·83	2·42	0·33	3·40	1·80
	Irrad. .	56·61	60·30	72·40	62·60	61·00	58·00	59·40	56·14	57·10	67·30	61·09±1·64
11	Control .	1·84	2·10	0·40	1·32	0·84	0·00	0·85	0·57	0·98	0·40	0·93
	Irrad. .	1·37	15·80	1·20	0·14	4·70	2·10	1·59	0·50	8·30	5·40	4·11±1·53

however, if the non-hatching percentages contained a large number of unfertilised eggs. Day 11 shows a drop to a value just slightly higher than the control level.

As stated previously, the dominant lethal incidence was followed mainly as a check on the running conditions of the experiment, but also fully confirms earlier observations.

(ii) *Deleted X's*

Table 2 gives the hyperploid frequency obtained over the four sampling days following 1000r X-rays. These data, though not very

TABLE 2

Percentage Hyperploidy following 1000r *X-rays to males*

Day	2	5	8	11
Total *y v f car* ♀♀ . . .	2532	1344	286	1011
Total hyperploid ♀♀ . .	3	3	6	0
Per cent. hyperploids . .	0·12	0·22	2·10	0·00
SE { upper limit . . .	0·24	0·45	3·32	0·18
{ lower limit . . .	0·06	0·10	1·22	0·00

extensive, fully confirm the peak incidence at day 8 found earlier (Bateman, 1957). The results are expressed graphically in fig. 1*b*.
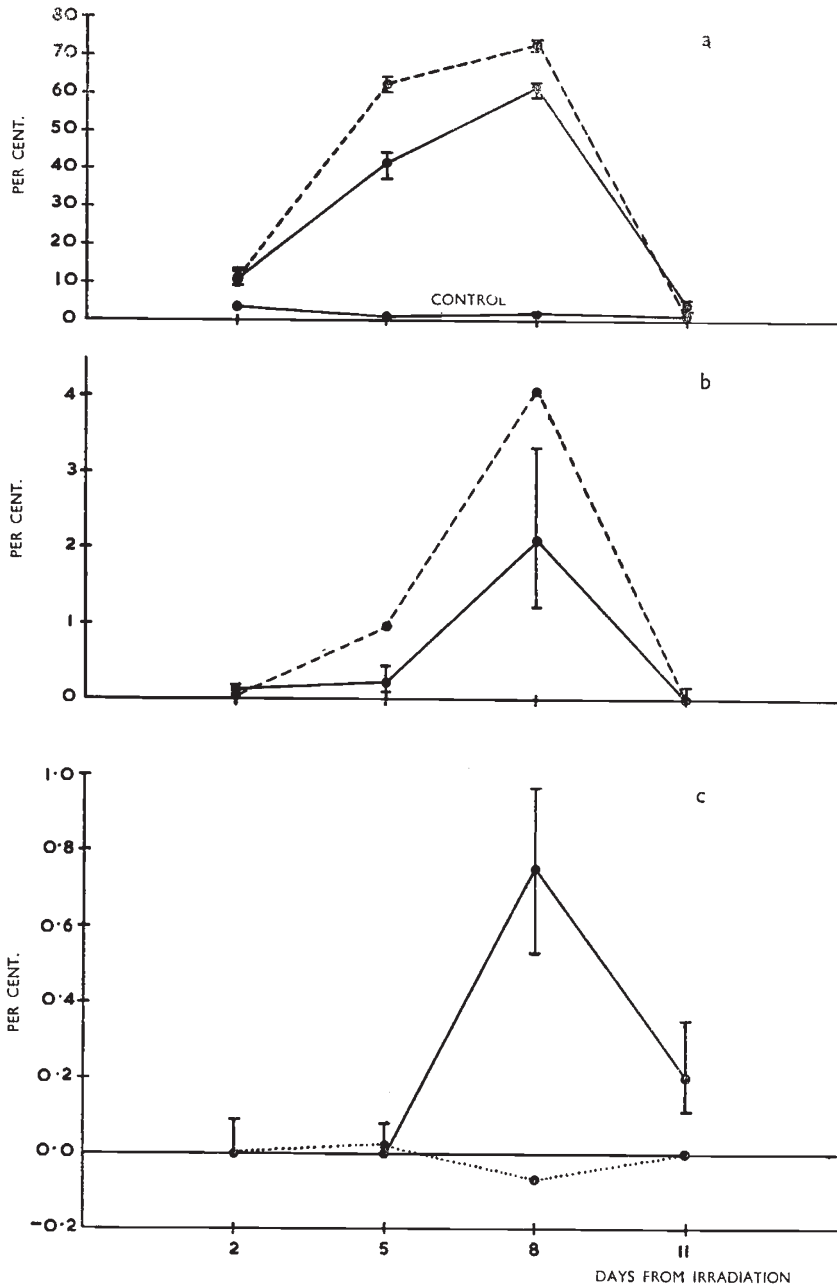
FIG. 1.—Variation in the rate of (a) Dominant lethals, (b) Hyperploids and (c) Cross-overs and " Mutations " on days 2, 5, 8 and 11 following 1000r X-rays. In graphs (a) and (b) continuous lines represent present data.   Broken lines represent data of Bateman (1956, 1957). In graph (c) continuous line represents per cent. cross-overs and dotted line represents per cent. " mutation " from present data.

Standard errors are represented by vertical lines.

(iii) *Autosomal recessive lethals*

The data on the frequency of recessive autosomal lethals are given in table 3 and fig. 2*a*.

The highest mutation rate was found on day 5 (12·19 per cent.). On day 8, the day showing the highest rate of both dominant lethals and deleted X's, the level of autosomal lethals was definitely lower (7·95 per cent.), indeed half-way between the values for day 5 and day 11, (3·82 per cent.) which showed a mutation rate even lower than day 2 (5·12 per cent.).

Analysis of the data obtained on the four days showed a highly significant variation between days. The standard errors were calculated

TABLE 3

Percentage autosomal and sex-linked recessive lethals following 1000r X-rays to males

Day	2	5	8	11
Autosomals				
Total . . .	781	607	566	680
Lethal . . .	40	74	45	26
Per cent. lethal .	5·12±0·79	12·19±1·33	7·95±1·14	3·82±0·74
Sex-linked				
Total . . .	944	617	440	731
Lethal . . .	19	35	15	9
Per cent. lethal .	2·01±0·46	5·67±0·93	3·41±0·87	1·23±0·41
Ratio autosomal/ sex-linked . .	2·5	2·1	2·3	3·1

from the total sample sizes on each day assuming homogeneity between successive runs of the experiment.

The results are in agreement with those of Auerbach (1954) who found a significant increase in mutation rate following 2000r X-rays, in the second brood, that is matings 4-7 days after treatment. In the tests for autosomal lethals however, Auerbach found excessive sterility in the treated males in the second brood. This she attributed to a speeding up of the rate of sperm release by the use of *Cy L* females, so that treated spermatocytes were already being utilised in the second brood. In all our matings, however, this period of sterility was confined to the single day 8. The difference may be due to the different dose levels (2000 and 1000r respectively).

(iv) *Sex-linked lethals*

The frequency of sex-linked recessive lethals is shown in table 3 and fig. 2*b*. There is a striking parallelism with the variation in autosomal lethal incidence. Statistical analysis of the results showed a highly significant variation between days with no detectable heterogeneity

between runs. The standard errors were based on the total sample sizes for each day. The reduced yield of sex-linked lethals from later matings of irradiated males has generally (*e.g.* Auerbach, 1954, Khishin,
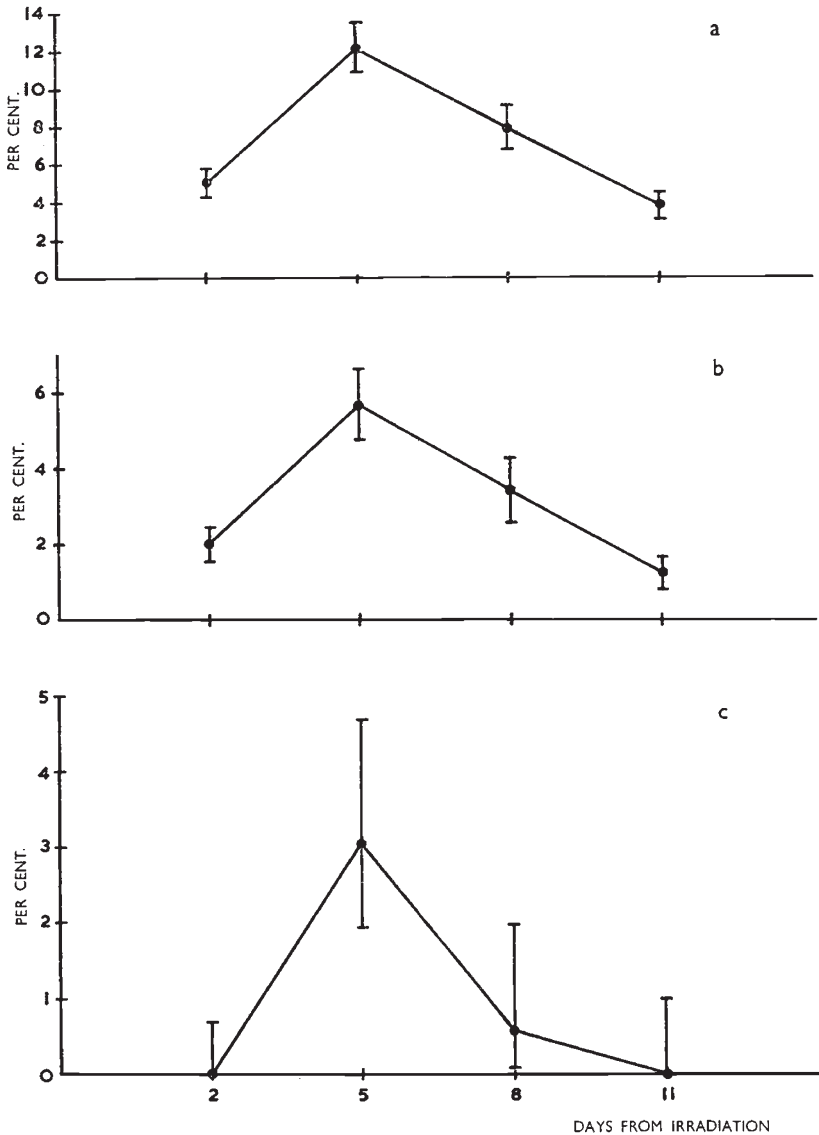

FIG. 2.—Variation in the rate of (*a*) Recessive autosomal lethals (*b*) Recessive sex-linked lethals and (*c*) Translocations on days 2, 5, 8 and 11 following 1000r X-rays.

Standard errors are represented by vertical lines.

1955) been attributed to germinal selection eliminating spermatogonia hemizygous for lethals. The dose similarity, however, between the results obtained for autosomal and sex-linked recessive lethals on all the sampling days, and emphasised by the ratio of mutation rates shown

in the bottom row of table 3, suggests that germinal selection cannot be the main factor. This must be reduced sensitivity of spermatogonia to the induction of mutations. There is, however, a suggestion that the ratio of autosomal to sex-linked lethals is higher on day 11 than previously, so *some* germinal selection may be operative. It is also possible that germinal selection would have been more marked in matings later than day 11. It is clear, however, that the main cause of reduction in yield of mutations from day 5 to day 11 and also the less spectacular reduction from day 2 to day 11 is due to variation in sensitivity to the mutagen, affecting autosomal and sex-linked lethals to similar extents.

(v) Translocations

Table 4 and fig. 2c show the translocations recovered on the four sampling days following 1000r. Although very few translocations

TABLE 4

Percentage translocations following 1000r X-rays to males

Day	2	5	8	11
Total	303	262	171	193
Translocations . .	0	8	1	0
Per cent. translocations .	0·0	3·1	0·6	0·0
SE { upper limit . .	0·7	4·7	2·0	1·0
{ lower limit . .	0·0	2·0	0·1	0·0

were recovered the results indicate a greater sensitivity for day 5 than any other day. These data are, therefore, consistent with the recessive lethal data rather than with the other structural changes (dominant lethals and deleted X's).

(vi) Induced crossing-over

Estimates of the rate of induced crossing-over were made over days 2-13. The results obtained are set out in table 5 and fig. 1c and fig. 3. The intention was to provide a means of identifying the specific stages of germ cell development at irradiation corresponding to each day's mating. As crossing-over can only occur in diploid cells, any cross-overs induced will indicate spermatogonia or spermatocytes at irradiation.

The map distances based on crossing-over in the female are *b-pr* 6·0 units, *pr-vg* 12·5 units. In the analysis of cross-over data using three markers, Auerbach (1954) has distinguished two cross-over classes: animals showing a single recessive marker (r_1) and those showing two recessives (r_2). Each r_2 phenotype has a complementary r_1 phenotype which should be produced by crossing-over with

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TABLE 5a

Frequency of crossing-over and " mutation " in daily samples from males given 1000r X-rays

Day	2	3	4	5	6	7	8	9	10	11	12	13
Non C-O	4240	4675	4256	4576	4616	3895	2919	3966	4713	4876	4293	3717
r_1	0	3	1	1	8	10	9	7	9	5	12	2
r_2	0	3	0	0	8	3	11	6	9	5	12	3
Per cent. C-O	0·00	0·00	0·00	0·00	0·04	0·16	0·75	0·30	0·38	0·20	0·56	0·16
Per cent. " mutation "	0·00	0·06	0·02	0·02	0·15	0·18	−0·07	0·03	0·00	0·00	0·00	−0·03

TABLE 5b

Overall frequency of crossing-over and " mutation " for the three periods—days 2-5, days 6-7, days 8-13

	2-5	6-7	8-13
Non C-O .	17,747	8,511	24,484
r_1 .	5	18	44
r_2 .	0	4	46
$2r_2$.	0	8	92
$r_1 - r_2$.	5	14	−2
Per cent. C-O .	0·00	0·094	0·374
Per cent. " mutation " .	0·028	0·164	−0·008

equal frequency. When, therefore, there is an excess of r_1 over r_2 one must suspect causes other than true crossing-over. Apart from crossing-over, r_2 can only be produced by back-mutation at the third locus or deletions large enough to include two loci. On the other hand, r_1 can be produced not only by mutation at that locus (including lethal mutations) but also by small deletions. It follows that r_2 can be taken as a much more reliable estimate of crossing-over than r_1. In the table, therefore, the " cross-overs " have been used to estimate two parameters: $2r_2$ or the true cross-overs, and $(r_1 - r_2)$, the mutations and deletions collectively referred to as " mutations ". The results of this analysis are shown in fig. 3. For comparison with the other data, fig. 1c shows the values on days 2, 5, 8 and 11 only.
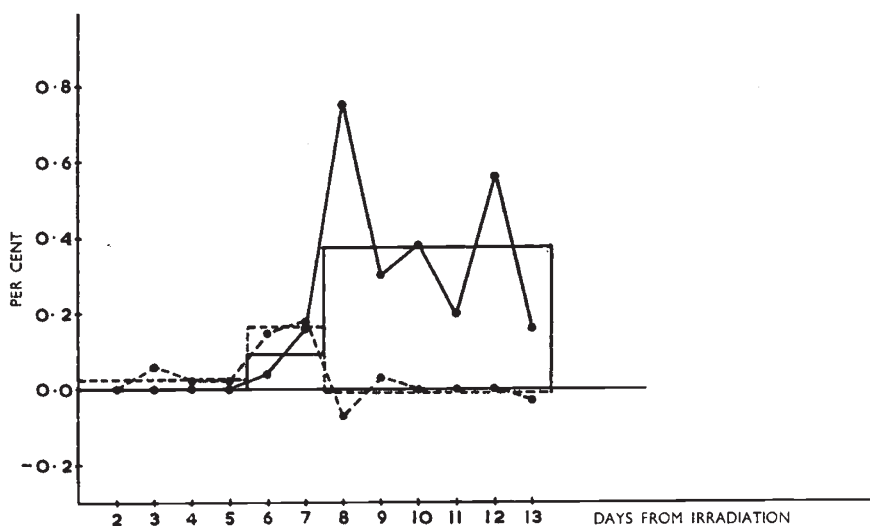

FIG. 3.—Variation in the rate of cross-overs and " mutations " in daily samples following 1000r X-rays. Continuous line equals per cent. cross-overs ($2r_2$). Broken line equals per cent. " mutations " ($r_1 - r_2$). Histograms show the mean level for cross-overs (continuous line) and " mutations " (broken line) over the three periods, days 2-5, 6-7 and 8-13.

The whole period surveyed can be divided into three qualitatively different stages: days 2-5 showing no crossing-over and a low level of " mutations "; days 6 and 7 showing a low level of crossing-over but the highest rate of " mutations " encountered, and days 8-13 during which crossing-over rises to a maximum and deletions are not evident.

Assuming that crossing-over can only occur in diploid cells it seems that days 2-5 represent irradiated sperm and spermatids, days 6-7 spermatocytes, and days 8-13 spermatogonia. The high sensitivity of spermatogonia to the induction of crossing-over is well established (Friesen, 1937, Auerbach, 1954, Sobels and van Steenis, 1957).

The reaction of spermatocytes as such does not appear to have been previously recorded. This stage, in which crossing-over is induced much less readily than in spermatogonia seems particularly susceptible, however, to the induction of small deletions.

2A

Further evidence that many of the r_1 class are not cross-overs is shown by a more detailed analysis. By crossing-over in heterozygotes for b pr vg, pr animals must be double cross-overs. These would be an extremely rare class, considering the infrequency of even single cross-overs. In all, there were 10 pr flies and none of the reciprocal class b vg. They occurred in spermatids, spermatocytes and spermatogonia and must represent either mutations or deletions. Out of the total of 100 genuine cross-overs $(2r_2)$, 90 occurred in the segment pr-vg and only 10 in the segment b-pr. Of the putative deletions (r_1-r_2) the loci b and pr had 10 each, but vg had none (estimated as -3).

In fig. 3 there is a suggestion of alternately high and low values for crossing over from day 8 onwards. A χ^2 test for heterogeneity on this part of the data, gave a value of $21\cdot40$ for 5 degrees of freedom, which has a probability of $0\cdot001$. The biggest contribution to this was $7\cdot99$ for day 8, which may represent a special category of germ cell, either just before or just after the last spermatogonial mitosis. In view of the fact that this is the day showing the greatest reduction in fertility after irradiation, there might be one cell stage which is completely eliminated and the mutations observed are from stages on either or both sides of it. In the latter case stages with contrasting sensitivities might be occurring in the same day's sample. Or again, the sperm tested may be from the few survivors of the stage which is most readily sterilised by X-rays. By analogy with the mouse (Oakberg, 1955) this would be the last generation of spermatogonia.

On removing day 8 there is still considerable heterogeneity $(\chi^2_{(4)}=13\cdot98)$ with a probability of $0\cdot01$. Perhaps this reflects the variation in sensitivity during each successive mitotic cycle.

4. DISCUSSION

The attempt to find other categories of mutation which would repeat the sensitivity pattern of either dominant lethals or deleted X's has evidently failed. On the other hand, sex-linked lethals, autosomal lethals and translocations show closely similar sensitivity patterns. This is especially so if one assumes that translocations, being proportional to a power of the dose greater than unity, would increase more rapidly than recessive lethals with increasing sensitivity of the cell stage.

The sensitivity pattern for dominant lethals up to day 8 could be reconciled with that for these other mutations if one assumed that a high proportion of unhatched eggs from day 8 matings were in fact unfertilised eggs owing to acute shortage of sperm. There is certainly a much lower rate of egg laying on that day (see Bateman, 1956) in a stock where virgin females lay only an occasional egg, so one can assume there is in fact a shortage of sperm. It remains to be established that the egg laying rate is imperfectly adjusted to the amount of sperm available so that when sperm is scarce the fertilisation rate is lowered—

or alternatively that egg laying is proportional to the total sperm, which on day 8 includes a high proportion lacking the capacity for fertilisation.*

In comparing the sensitivity patterns for various types of mutation, allowance must be made for the fact that chromosome rearrangements of whatever kind are never recovered from irradiated spermatogonia. This applies to dominant lethals (*e.g.* Bateman, 1956), deleted X's (Bateman, 1957), translocations and inversions (Ward and Alexander, 1957). In the case of translocations and inversions which are fully viable this cannot be due to the elimination of spermatogonia with cell lethals, though such an explanation could apply to dominant lethals. Either the primary incidence of chromosome breakages in spermatogonia is low, or, with a normal incidence of breakages, *rearrangements* are inhibited, so that all breaks that fail to restitute lead to dominant lethals, which behave as cell lethals in spermatogonia.

Some rearrangements have been observed in *Microtus* spermatogonia by Ockey (1959) following TEM treatment but they were at a very low incidence and all those identified were confined to the sex chromosomes, which are a special case being overwhelmingly heterochromatic.

It follows that in comparisons of sensitivity patterns between rearrangements and mutations, differences for day 11 can be taken for granted. It is the differences over days 2, 5 and 8 which should receive our attention. Over this period, recessive lethals, both autosomal and sex-linked, and translocations show the same pattern. There is a sharp rise from day 2 (sperm) to day 5 (spermatids) followed by a fall on day 8 (spermatocyte or late spermatogonia?) to an intermediate sensitivity. Deleted X's are exceptional with a very different pattern. The rise from day 2 to day 5 is followed by a rise to day 8 which is proportionately the same but in absolute terms is much greater.

What is there special about deleted X's which separates them from the rest? It cannot be that they involve the sex chromosomes (c.f. sex-linked lethals), nor that they are structural changes (c.f. translocations). They have two distinct features, they are *intra*-changes and they are deletions.

In the Drosophila spermatocyte the nucleus is very large in comparison with the amount of chromatin it contains. The bivalents will be widely separated as can be confirmed cytologically in stages where the chromosomes are visible. This could explain why any rearrangements would tend to be intra-changes. Inversions have not been scored in this material. Deletions from the autosomes are lethal when large and when small are not readily detected. However, our data on induced crossing-over are valuable here. The excess of r_1 over r_2 was confined to days 6 and 7, that is to spermatocytes, and it has already been argued that this is probably due to small deletions. We therefore propose that deletions are a mutagenic response more readily induced in spermatocytes than elsewhere. The unique sensitivity pattern of

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* *Note added in proof.* We have now verified that in spite of the shortage of sperm, the fertilisation rate on day 8 is normal: thus the high dominant lethal rate is *genuine*.

deleted X's is therefore explained as follows. The variation in post-meiotic germ cells is governed by the general variation in sensitivity to mutagens affecting all kinds of mutational response. Spermatocytes, however, show a special propensity to induced deletions. Owing to the special condition of this type of cell, the most obvious hypothesis is that these deletions are produced by non-homologous crossing-over either between chromosomes or between sister chromatids.

There is one difficulty remaining. The peak incidence of deleted X's is on day 8, whereas the small deletions in our cross-over data were only detected on days 6 and 7. On day 8 however, there was an exceptionally high yield of true cross-overs (r_2) which by error variation could mask the presence of small deletions ($r_1 - r_2$).

5. SUMMARY

F_1 males irradiated with 1000r X-rays were mated on days 2, 5, 8 and 11 after treatment to different classes of female. The incidence of dominant lethals, hyperploids and translocations (structural changes), and recessive autosomal and sex-linked lethals (gene changes) was measured for each day.

Estimates of induced crossing-over in the *b pr vg* region, made in daily samples from day 2 to day 13, were used to identify specific stages of germ cell development. In addition, the excess of animals showing a single recessive marker over those showing two markers was used to estimate the proportion of " mutations " or small deletions.

The pattern of sensitivity was similar for recessive autosomal and sex-linked lethals and translocations, showing a rise from day 2 (sperm) to day 5 (spermatids) followed by a fall on day 8 (spermatocytes or later spermatogonia?).

Deleted X's showed a unique sensitivity pattern with a peak on day 8. This was attributed to the high sensitivity of spermatocytes to intrachanges and in particular to deletions.

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