THE RELATION BETWEEN RECESSIVE LETHALS, DOMINANT LETHALS, AND CHROMOSOME ABERRATIONS IN *DROSOPHILA*

By D. E. LEA and D. G. CATCHESIDE

The Strangeways Research Laboratory, and the Botany School, Cambridge

(With Three Text-figures)

1. Introduction

It has become clear in recent years that neither the recessive lethals nor the dominant lethals in *Drosophila melanogaster* form a single class, and that both are connected to some extent with structural changes in chromosomes. With some of the recessive lethals, which (following Fano, 1941) we shall refer to as type A, no structural change can be detected in the salivary gland chromosomes, and it is reasonable to consider these as changes in single genes. Others (type B) show deletion of from 1 to 50 bands of the salivary chromosome, and in these instances the lethal is to be attributed to the absence of one or more genes. Others (type C) show gross structural changes—inversions or interchanges (reciprocal translocations)—often without any deletion as far as can be observed in the salivary gland chromosomes. When lethals at known loci are studied as in Demerec's extensive observations of Notches (Demerec & Fano, 1941), it is found that gross structural changes which give rise to lethals involve a break at or immediately adjacent to the locus at which the lethal appears. As pointed out by Demerec (1939) (see also Muller & Altenburg, 1930), there are two possible explanations of this association between a recessive lethal and a chromosome break. One is that the ionizing particle which causes the break also, in some instances, causes a lethal mutation in the gene through which, or close to which, it passes. The other explanation is in terms of the *position effect* and is that the behaviour of the gene is changed when it is transferred to a different position in the chromosome set, either because it has been separated from the gene to which it is usually adjacent, or because it is brought adjacent to a different gene. Of recent years the general tendency appears to be to accept the position-effect explanation. There does not seem, however, to be much justification for this choice between the two alternatives. That a position effect is a frequent accompaniment of chromosome interchange involving the heterochromatin is certain. There are not very many established cases of position effect not involving heterochromatin, and while some instances of visible mutations due to this cause have been established, there is little reason to suppose that a large proportion of recessive mutations are so produced. Demerec (1937), for example, in thirty visible mutations at ten different loci did not find one connected with a gross structural change. It is a considerable assumption therefore on the basis of present knowledge to ascribe to position effect some 30 or 40% of all the sex-linked lethals induced at 3000 r.

There is one way in which a test between the two explanations can be made. It is known that the yield of gross structural changes increases more rapidly than the first power of the dose (approximately as the 3/2 power of the dose between 1000 and 4000 r.). This has been established for the structural changes associated with lethals (Oliver, 1932) as well

as for structural changes not so selected (Bauer, Demerec & Kaufmann, 1938; Muller, Makki & Sidky, 1939), and is due to the fact that a gross structural change involves two independently produced breaks. The 3/2 power law has also been established for two known position effects, namely, cubitus interruptus and mottled white (Muller, 1940, in contradiction however to a first-power law found by Khvostova & Gavrilova, 1935, 1938). The lethals not associated with gross structural change, namely, gene changes and minute deletions, will increase in proportion to the dose. On the position-effect explanation therefore the total observed yield of lethals will be the sum of two terms, one proportional to dose, and the other to the 3/2 power of dose. The dose curve should therefore rise more rapidly than the first power of the dose.

On the alternative explanation that the lethals associated with chromosomal changes are gene changes, the *total* number of lethals should be proportional to dose. The essential difference is that on this explanation there is a lethal at a certain proportion of the breakage points irrespective of whether these particular breaks restitute or take part in interchange. On the position-effect explanation there is a lethal at a breakage point only if the break takes part in a chromosome interchange.

When this test is applied (see next section) the evidence is against the position-effect hypothesis.

In view of this result we have pursued the implications of the alternative explanation, that a lethal associated with a gross structural change is due to mutation or deletion of a gene at, or immediately adjacent to, the breakage point. On this view it appears likely that lethals *not* apparently associated with any chromosome change are restitutions, i.e. a break has occurred and the broken ends have rejoined in the original formation. We are led to estimates of the frequency with which breaks are primarily produced, the frequency of restitutions, and the proportion of breaks at which a lethal is simultaneously induced.

It has been realized for some time that chromosome interchanges which result in dicentric and acentric chromosomes must behave as dominant lethals, since such formations are not found in salivary chromosomes and there is no reason to believe that they do not occur following irradiation of the sperm. It has also been realized (Fano, 1941) that the frequency of these aberrations as inferred from the frequency of the viable types of chromosome aberrations, is not sufficient to account for the total number of dominant lethals observed. More recently (Muller, 1941; Pontecorvo & Muller, 1941; Pontecorvo 1941, 1942), it has been shown that chromosome breakage, not followed by interchange with other breaks or by restitution, but probably by sister-union of the chromatids when the chromosome divides, probably accounts for the remaining dominant lethals. Apparently a certain proportion of the chromosome breaks neither restitute nor interchange, but instead behave as dominant lethals. It is not clear what determines this choice; it suffices for our further argument if we ascribe a value p to the probability that a break shall neither restitute nor interchange, and a value $q = 1 - p$ to the (combined) probability that it will either restitute or interchange. If r chromosome breaks are primarily produced in a cell, q^r can plausibly be supposed to be the chance that *all* either restitute or take part in interchange. On the assumption of random union between broken ends, it is possible to arrive at formulae (see § 3) for the number of dominant lethals as a function of the dose, and for the proportion of viable sperm having chromosome aberrations. Comparison of these formulae with experiment enables p to be determined,

the value found being 0·24. It also enables an estimate to be made of the number of breaks primarily produced in the sperm by a given dose.

We have thus obtained two estimates of the number of chromosome breaks primarily produced in the sperm, one based on our analysis of the recessive lethals, and one on the independent basis of dominant lethals and structural changes. The fact that these estimates are in satisfactory agreement supports our analysis.

2. Recessive lethals

Extensive data exist on the yield of recessive lethals as a function of dose. These afford no evidence of an increase with dose more rapid than the first power of the dose. To make the test objective, we have proceeded as follows. Assuming a formula $m = \alpha D + \beta D^{1.5}$ for the mean number of lethals per sperm produced by dose D, which is the formula expected on the position-effect interpretation, we have fitted by the least squares method the experimental data for various relative values of the coefficients α and β. By the χ^2 test the goodness of fit of the theoretical curve to the data has been tested.

Since the observation is not of the mean number of lethals per sperm, but of the proportion of sperm carrying one or more lethals, the data, after correction for spontaneous lethals have to be fitted to the formula $1 - e^{-m}$ (cp. Zimmer, 1934). The experimental data we make use of are those accumulated over a number of years by Timoféeff-Ressovsky (1939), based on some 60,000 cultures, and are given in Table 1 together with the results of the χ^2 tests. It is evident that these data provide no evidence for any (dose)$^{\frac{1}{2}}$ class,

Table 1. *Sex-linked lethals as a function of dose (experiments of Timoféeff-Ressovsky)*

Dose (r.)	Sperm tested	Lethals	Lethals per sperm (%)
0	32140	63	0·19 ± 0·02
1500	15281	649	4·25 ± 0·16
3000	11738	1027	8·75 ± 0·26
6000	9116	1462	16·04 ± 0·38

Postulated proportion of lethals belonging to (dose)$^{\frac{1}{2}}$ class at 3000 r.	χ^2	D.F.	P
0	2·3	2	0·32
12·5 %	3·6	2	0·16
17·5 %	6·4	2	0·04
22·0 %	9·6	2	0·008

make it improbable that the lethals of this class, if it exists, constitute as much as 17·5 % of the total number of lethals at 3000 r., and practically exclude the possibility of the proportion being as high as 22 % at 3000 r.

Turning now to the experimental data concerning the proportion of sex-linked recessive lethals which, at 3000 r., are associated with gross structural change, we find the following estimates in the literature. Oliver's (1932) experiments using random lethals gave $15/61 = 25 \pm 6$ %. Demerec's (1937) experiments with random lethals gave $5/16 = 31 \pm 12$ %. Some further data of Demerec (1937) involving lethals at eighteen selected loci gave $24/61 = 39 \pm 6$ %, while his observations (Demerec & Fano, 1941) confined to a single known locus (Notch) gave $34/85 = 40 \pm 5$ %. The average of all these estimates, which are reasonably consistent, is 35 ± 3 %. This proportion being higher than the maximum proportion which can be reconciled with Table 1, we conclude that the lethals associated with gross structural change do not constitute an *additional* class, but represent those

cases where the ionizing particle which caused the lethal also caused a break in its passage through the chromosome, which break took part in a gross structural change. We develop the further discussion on this basis.

There is good reason to believe (Muller, 1941) that not all the chromosome breaks primarily produced take part in structural change, but that often the broken ends rejoin and the restituted chromosome is cytologically indistinguishable from an unbroken chromosome. Whether restitution or structural change occurs appears not to depend on a difference in the breakage process, but mainly on whether other breaks are available with which interchange can occur. It is necessary (on our interpretation) to accept that since some of the breaks which take part in structural change are lethals, so also some of the breaks which restitute are lethals. Such lethals will be recorded as type A lethals (p. 10), i.e. lethals without any cytological detectable chromosome change.

We have no *a priori* reason to believe that a type A lethal cannot be produced without the chromosome at the same time being broken. However, admitting the necessity, on other grounds, for a considerable number of restitutional breaks, a large part of the type A lethals must be restitutional breaks. We shall see how far a consistent picture can be obtained on the basis that *all* the type A lethals are restitutional breaks. (Some evidence in favour of this hypothesis is given on p. 14.)

As a beginning we need to know the numbers of the different types of lethals produced by a given dose, say 3000 r. Taking the yield of lethals to be 2·9 % per 1000 r., we shall have 87 lethals per 1000 chromosomes for a dose of 3000 r. 35 %, i.e. 30, will be type C, involving gross structural change. The remaining 57 will be types A and B. Information on the relative proportion of types A and B is available from the observations of Slizynski (1938, 1942). (Data by Demerec on the relative numbers of deficient and non-deficient lethals at *selected* loci give a larger proportion of deficiencies, for a reason which is explained on p. 14. Here we require the proportion at *random* loci.) Slizynski made an examination of salivary gland chromosomes containing lethals and found that the proportion of lethals associated with minute deficiencies was in one experiment 4 out of 13, and in another 2 out of 6. Taking the proportion therefore to be $6/19 = 0{\cdot}32$, we infer that there are $57 \times 0{\cdot}32 = 18$ type B and therefore 39 type A lethals. Thus 3000 r. produces in 1000 *X*-chromosomes 87 lethals, of which 39 are type A, 18 are type B, and 30 are type C.

Now the number of breaks in the euchromatin of the *X*-chromosome which take part in gross structural change when a dose of 3000 r. is given to the sperm is 80 per 1000 *X*-chromosomes (deduction by Fano (1941) from salivary gland observations of Bauer (1939*b*)). Of these 30 carry lethals (namely, the 30 type C lethals). Evidently the probability that a chromosome break shall cause a lethal is $30/80 = 0{\cdot}38$.

There are 18 minute deficiencies (i.e. the type B lethals) which are lethal because one or more loci are deleted. There is reason for believing that when a chromosome is broken in two places, the probabilities are approximately equal that the segment between the breaks shall be deleted and that it shall be inverted. We presume therefore that there are also 18 minute inversions. An inversion will not, in our view, behave as a lethal *per se*, but since it involves two breaks, each of which has a probability of 0·38 of being a lethal, the probability is $1 - (1 - 0{\cdot}38)^2 = 0{\cdot}62$ that at least one of the breaks will be a lethal. Thus of the 18 minute inversions $18 \times 0{\cdot}62 = 11$ will behave as lethals, and will therefore be included in the type A lethals, minute inversions not being sufficiently certainly recognizable to be put into a separate class.

This leaves 28 type A lethals which are restituted breaks. Since only 38% of breaks are lethals, the total number of restituted breaks must be $28/0{\cdot}38 = 74$.

The total number of breaks of all sorts produced by 3000 r. in the euchromatin of 1000 *X*-chromosomes is therefore 226, made up of 36 in minute deletions (two breaks per deletion), 36 in minute inversions (two breaks per inversion), 80 in gross structural changes, and 74 which restitute. The rate of production of primary breaks per *X*-chromosome by 3000 r. is thus 0·226. Of the 226 breaks at 3000 r., a proportion, $80/226 = 35\%$, take part in gross structural change. At greater doses the proportion will be higher, at smaller doses it will be lower.

It is to be noted that 11 out of 39, or about 30%, of the type A lethals are expected to be minute inversions. Slizynski, from examination of the salivary chromosomes, suspected that some of the non-deficient lethals were minute inversions.

It is also to be remarked that there are 18 minute deletions to 74 restitutional breaks, a ratio of 1 to 4. Now in her cytological study of inversion breakage points Hoover (1938) found that there were 5 breakage points with deletion to 15 breakage points without deletion, a ratio of 1 to 3. The agreement between these ratios suggests that we have not seriously overestimated the number of restitutional breaks by assuming that *all* 'point' lethals are restitutions, and supports the assumption which we made on p. 13. Doubtless there are a few point lethals which are not restitutional breaks, but at the present stage this further subdivision of the lethals is not profitable.

Demerec (1939; also Demerec & Fano, 1941) has collected a considerable amount of information concerning the production of recessive lethals at selected loci in the *X*-chromosome, particularly the locus Notch (band 3C7 in the salivary gland map). Among 85 independently originating Notches occurring in some $7{\cdot}4 \times 10^5$ *X*-chromosomes irradiated by 2500–3000 r., there were 34 gross structural changes having a break adjacent to the 3C7 band, 37 deficiencies of various sizes which included this band, and 11 Notches without any cytologically detectable structural change. It is to be observed that in this collection of lethals at a *selected* locus there are many more type B (deficient) than type A (non-deficient) lethals, in contrast to Slizynski's results with random lethals (p. 13). Further, nearly half (18/37) of the deficiencies are of 10 bands and upwards, whereas Slizynski's were all smaller. The explanation is that large deficiencies are really less frequent in the salivary chromosomes than small, but when it occurs a large deficiency is more likely to include a *specified* locus than is a small deficiency. To deduce from Demerec's data the number of deficiencies of different sizes we can proceed as follows, on the basis of the assumption that breaks are equally probable anywhere in the euchromatin, so that the probability that a deficiency of x bands shall contain a specified band is $x/647$, 647 being the number of bands in the *X*-chromosome (counting a doublet as one, Demerec & Fano, 1941). Thus if n deficiencies of x bands including the Notch locus are observed, we interpret this to mean that about $647n/x$ deficiencies of about this size are produced in the whole chromosome. In this way the numbers of deficiencies of different sizes induced by 2500–3000 r. per 1000 *X*-chromosomes can be deduced from Demerec's data, and are set out in Table 2.

Table 2. *Distribution of sizes of deficiencies*

No. of bands deficient	1	2	3–5	6–10	11–15	16–20	21–30	31–40	Total
No. of deficiencies of this size per 1000 chromosomes	7·87	2·19	0·76	0·47	0·37	0·13	0·14	0·08	12·01

The total number of deficiencies, 12 per 1000 *X*-chromosomes for a dose of 2500–3000 r., is in fair agreement with the estimate of 18 for a dose of 3000 r. deduced from consideration of deficiencies associated with random lethals.

Demerec found in his collection of Notches three which were deficiencies which did not *include* the band 3C7 but had one break *adjacent* to it. In this case presumably the break adjacent to Notch caused the lethal. Allowing as usual that the probability of a break causing a lethal is 0·38, it follows that there were in all $3/0{\cdot}38=8$ deficiencies having a break adjacent to Notch. A deficiency involves two breaks, and the probability that a deficiency located at random in the chromosome shall have one of its breaks adjacent to the Notch band, without the deficiency including this band, is clearly 2/647, 647 being the total number of bands. Thus the total number of deficiencies is deduced to be $8\times 647/2=2588$. This is the number of deficiencies in $7{\cdot}4\times 10^5$ *X*-chromosomes, the number per 1000 *X*-chromosomes therefore being 3·5. This estimate is smaller than the number 12 obtained above (Table 2). The explanation may simply lie in the smallness of the numbers involved, or it may mean that a deficiency of a single band is not produced by two distinct breaks, but by another mechanism, e.g. a single gene being rendered incapable of duplication. The number of deficiencies for *more* than one band given in Table 2 is 4·1, in good agreement with 3·5.

On our view, according to which the probability of a break having a lethal associated with it does not depend on what sort of arrangement the break enters into, we should expect interchanges involving heterochromatin to constitute only a small proportion of the total number of structural changes involving a lethal at a given locus, since heterochromatin is concerned in only about one-sixth of observed chromosome breaks (Bauer *et al.* 1938). This is borne out in Demerec's (1939) observation of recessive lethals at the Notch locus, where only 1 out of 10 lethals associated with structural changes involved heterochromatin (excluding from consideration a case involving a deficiency as well as gross structural change). The breakage point was adjacent to the 3C7 band. On the position-effect interpretation, we might have expected lethals associated with heterochromatic aberrations to have been particularly frequent, and for the range of the effect in cases involving heterochromatin to have extended to greater distances from the locus of the break, by analogy with known position effects.

We have thus been able to build up a consistent picture of the production of recessive lethals on the basis that a certain proportion (38%) of the primary breaks in the *X*-chromosome have a lethal at the breakage point, the lethal being produced by the ionizing particle which caused the break, and not being dependent for its expression on the break taking part in chromosome rearrangement.

3. Dominant lethals and structural changes

We take p to be the probability that a given break shall neither restitute nor take part in interchange but instead give rise to a dominant lethal effect, and $q=1-p$ the probability that it shall either restitute or take part in interchange. If r primary breaks are produced in a given sperm, q^r is the probability that all shall either restitute or take part in interchange. In considering the rejoining process we shall adopt the assumptions and simplifications made by Catcheside (1938), namely, omit consideration of interchanges between breaks in the same chromosome arm and consider only interchanges between different chromosome arms, and secondly, assume that rejoining between broken ends is

at random. The first simplification will not be a serious source of error; the second requires justification. In *Tradescantia* pollen grains such an assumption would be completely misleading, since there a break has a much higher chance of restituting than of interchanging with other breaks in the nucleus, and two breaks have a very small chance of interchanging unless the breaks are produced at a separation much smaller than the nuclear diameter (Lea & Catcheside, 1942). But in *Drosophila* sperm the conditions are different, since the rejoining process takes place after the sperm has entered the egg (Muller, 1940), and it is quite probable that the spatial distribution of the breaks when rejoining is occurring bears little relation to their spatial distribution at the moment of their production. We shall therefore assume that in *Drosophila* sperm, in contrast to *Tradescantia* pollen grains, rejoining is sufficiently nearly random.

Suppose that with dose D the mean number of breaks per sperm is $m=\alpha D$. The proportion of sperm having r breaks per sperm is given by the Poisson distribution, and is $e^{-m}\,m^r/r!$. The probability that a sperm shall have no breaks is e^{-m}. The probability that it shall have one break is me^{-m}. Sperm with one break will contribute $(1-q)\,me^{-m}$ to the number of dominant lethals, and qme^{-m} to the number of viable nuclei without aberrations.

Of the $\frac{1}{2}m^2e^{-m}$ sperm with two breaks per sperm, $(1-q^2)\,\frac{1}{2}m^2e^{-m}$ will be dominant lethals owing to failure of one or both breaks either to restitute or to interchange. In $\frac{1}{2}m^2q^2e^{-m}$ sperm the four broken ends will all join. Under the assumption of random joining, in one-third of these sperm there will be restitution, giving viable sperm without aberrations, in one-third there will be symmetrical interchange giving viable sperm with chromosome aberration, and in one-third there will be asymmetrical interchange adding a further quota to the dominant lethals. Thus of the sperm with two breaks, $\frac{1}{6}m^2q^2e^{-m}$ will be viable without aberration, $\frac{1}{6}m^2q^2e^{-m}$ will be viable with aberration, and the remaining $\frac{1}{2}m^2e^{-m}\,(1-\frac{2}{3}q^2)$ will carry dominant lethals.

In general there will be $e^{-m}\,m^r/r!$ sperm having r breaks. In $e^{-m}\,m^rq^r/r!$ sperm no breaks will remain unjoined. In a sperm of this class the r breaks can rejoin in $1.3.5....(2r-1)=(2r)!/(r!\,2^r)$ ways, of which one way is viable without aberration, $(r!-1)$ ways are viable with aberration, and the remainder are inviable (Catcheside, 1938).

Collecting the contributions from sperm with various numbers of breaks, and replacing m by its value αD, we have:

Proportion of cells which are viable and without aberration is $X=e^{-\alpha D}\,S_1$, where

$$S_1=1+\alpha qD+\tfrac{1}{6}\,(\alpha qD)^2+\ldots+\frac{(2\alpha qD)^r}{(2r)!}+\ldots. \tag{1}$$

Proportion of cells which are viable (with and without aberrations) is $Y=e^{-\alpha D}\,S_2$, where

$$S_2=1+\alpha qD+\tfrac{1}{3}\,(\alpha qD)^2+\ldots+\frac{(3\alpha qD)^r.\,r!}{(2r)!}+\ldots. \tag{2}$$

Total number of primary breaks formed in viable cells, per total sperm, is $Z=e^{-\alpha D}\,S_3$, where

$$S_3=\alpha qD+\tfrac{2}{3}\,(\alpha qD)^2+\ldots+\frac{(2\alpha qD)^r.\,r.\,r!}{(2r)!}+\ldots. \tag{3}$$

The sums S_1, S_2, and S_3 of the infinite series in equations (1), (2) and (3) can be evaluated

quite easily arithmetically, for small values of αqD, since the series converge rapidly. Algebraic expressions for them are more convenient for larger values of αqD. These are

$$S_1 = \cosh \sqrt{(2\alpha qD)}, \tag{4}$$

$$S_2 = 1 + \sqrt{(\tfrac{1}{2}\pi\alpha qD)}\, e^{\frac{1}{2}\alpha qD} \operatorname{erf} \sqrt{(\tfrac{1}{2}\alpha qD)}, \tag{5}$$

$$S_3 = \tfrac{1}{2}\alpha qD \left\{ 1 + \frac{1+\alpha qD}{\sqrt{(\tfrac{1}{2}\alpha qD)}} \frac{\sqrt{\pi}}{2} e^{\frac{1}{2}\alpha qD} \operatorname{erf} \sqrt{(\tfrac{1}{2}\alpha qD)} \right\}, \tag{6}$$

where $\cosh x = \frac{1}{2}(e^x + e^{-x})$ is the hyperbolic cosine, and $\operatorname{erf} x = \dfrac{2}{\sqrt{\pi}} \displaystyle\int_0^x e^{-x^2}\, dx$ is the error function. In Table 3 values of S_1, S_2, and S_3 are tabulated for a suitable range of values of αqD.

Table 3

αqD	S_1	S_2	S_3	$(1 - S_1/S_2)$	S_3/S_2
0·32	1·337	1·356	0·3952	0·0140	0·2914
0·50	1·543	1·592	0·6942	0·0309	0·4360
0·72	1·811	1·920	1·152	0·0572	0·5996
0·98	2·151	2·373	1·849	0·0935	0·7793
1·28	2·577	2·996	2·915	0·1396	0·9731
1·62	3·107	3·858	4·553	0·1945	1·180
2·00	3·762	5·060	7·090	0·2565	1·401
2·88	5·557	9·172	17·29	0·3941	1·885
3·92	8·253	17·78	43·23	0·5357	2·432
5·12	12·29	36·82	112·2	0·6663	3·046
6·48	18·31	81·57	304·6	0·7755	3·734
8·00	27·31	193·6	870·8	0·8590	4·497

Table 4. *Mean number of primary breaks per viable sperm*

Dose (r.)	1000	1500	2000	3000	4000	6000
Mean no. of primary breaks per viable sperm	0·49	0·69	0·89	1·23	1·54	2·15

One of the observable quantities is the proportion of viable sperm which have chromosome aberrations. The theoretical expression for this proportion is evidently $(1 - X/Y) = (1 - S_1/S_2)$, and is listed in Table 3 as a function of αqD. In Fig. 1 we show experimental data of the proportion of viable sperm with chromosome aberrations as a function of the dose as determined by Catcheside (1938) and Bauer *et al.* (1938), together with the theoretical curve $(1 - S_1/S_2)$, which has been fitted to the data by taking $\alpha q = 0{\cdot}57$ per 1000 r.

A second observable quantity is $(1 - Y)$, the proportion of total sperm which are non-viable. In Fig. 2 the experimental observations of Catcheside & Lea (1945*a*) on the proportion of eggs fertilized by irradiated sperm which fail to attain the adult stage are plotted, together with the theoretical curve. In computing the formula for Y (see equation (2)) we already know that $\alpha q = 0{\cdot}57$, which enables S_2 to be calculated for each dose with the aid of Table 3, α still remains arbitrary. The value $\alpha = 0{\cdot}75$ was found to give the best fit of the theoretical curve to the experimental points.

It follows that $q = 0{\cdot}57/0{\cdot}75 = 0{\cdot}76$, so that we have the figures:

$\alpha = 0{\cdot}75$ is the number of primary breaks produced per sperm per 1000 r.
$q = 0{\cdot}76$ is the probability that a break shall join, either in restitution or in interchange.
$p = 1 - q = 0{\cdot}24$ is the probability that a break shall remain unjoined, but instead shall behave as a dominant lethal.

It is with these values of α, p and q that the theoretical curves in Figs. 1 and 2 have been computed.

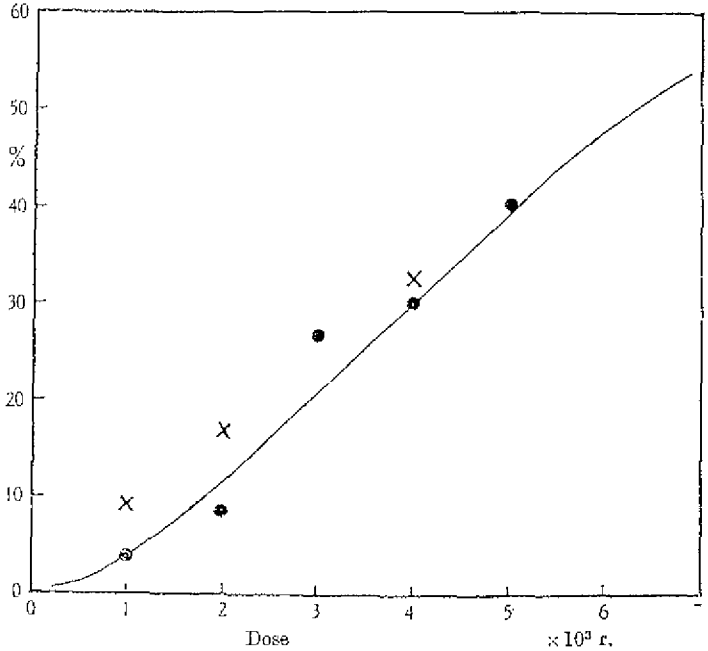

Fig. 1. Percentage of viable sperm having chromosome aberrations. Curve theoretical; points experiments of: ● Bauer *et al.* (1938), × Catcheside (1938).

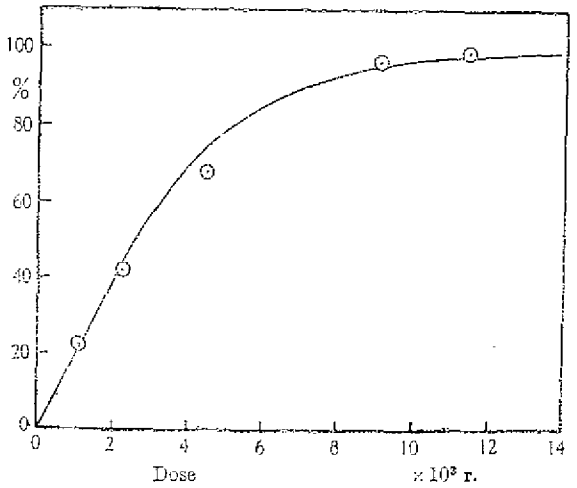

Fig. 2. Percentage of dominant lethals as a function of dose. Curve theoretical; points experiments of Catcheside & Lea (1945*a*).

It is of interest to calculate the mean number of breaks primarily formed per *viable* sperm (which will be a little less than αD). Referring back to equations (2) and (3), this is seen to be S_3/S_2. S_3/S_2 is tabulated against αqD in Table 3. Using the value $\alpha q = 0{\cdot}57$ just found, and interpolating in Table 3, we obtain the estimates given in Table 4 of the mean number of primary breaks per viable sperm.

We can calculate from these figures the number of primary breaks in the euchromatin

of the X-chromosome, by making use of the result that a fraction 0·162 of all observed breaks occurs there (Fano, 1941; based on data of Bauer, 1939*b*). Thus at 3000 r. $0{\cdot}162 \times 1{\cdot}23 = 0{\cdot}199$ primary breaks occur in the euchromatin of the X-chromosome.

4. Sex-ratio distortion

The theory of dominant lethals given in the previous section can be extended to cover experiments on the distortion of the sex ratio in the progeny of irradiated males. The ratio of females to males is reduced below unity on account of the fact that radiation-induced changes in the X-chromosome of an X-bearing sperm make a contribution to the total of dominant lethals in excess of the contribution made by changes in the Y-chromosome of a Y-bearing sperm. The distortion of the sex ratio is more marked when the irradiated males have the X^{c2} ring chromosome than when they have an ordinary rod-X-chromosome. The various types of change contributing to the distortion of the sex ratio have been discussed by a number of authors (Bauer, 1939*a*; Muller, 1940; Pontecorvo, 1941, 1942; Catcheside & Lea, 1945*a*, *b*), whose accounts should be referred to for the justification of the following statements.

The changes of principal importance in the distortion of the sex ratio are:

(*a*) *Breaks in the sex chromosome not taking part in interchange with other breaks.* Such breaks in a Y-chromosome, or a rod-X-chromosome, will be viable if they restitute, lethal (nearly always) if they do not restitute but instead undergo sister-union. The probability of failure to join is given (p. 17) by $p = 0{\cdot}24$.

Such breaks in a ring-X^{c2}-chromosome will similarly be (usually) lethal in a proportion $p = 0{\cdot}24$ of instances owing to failure to restitute. They will not all be viable, however, in the proportion $q = 0{\cdot}76$ of instances where restitution occurs, since in half of such cases restitution leads to an inviable chromosome (Catcheside & Lea, 1945*b*).

(*b*) *Breaks in the sex chromosome which take part in interchange with breaks in other chromosomes.* In the case of a rod-X- or Y-bearing sperm, such interchanges will be lethal if one or more dicentric or acentric chromosomes are formed, and will be viable if only symmetrical interchange occurs. In the case of a ring-X^{c2}-bearing sperm, *any* interchange involving the X^{c2}-chromosome and an autosome will be lethal.

In addition to these major contributions to the distortion of the sex ratio, there are minor ones which will be mentioned later; our calculation is limited to (*a*) and (*b*). The procedure is to carry out a dominant lethal calculation, such as we have given in § 3, separately for Y, for rod-X, or for ring-X^{c2}-bearing sperm. The calculation already given leading to formula (2) with $\alpha = 0{\cdot}75$ we shall take to apply to rod-X-bearing sperm. (Strictly all the experimental data used in determining the value of α should have been confined to X-bearing sperm. Of the data which were available and which are employed in Figs. 1 and 2, some referred to X-bearing sperm and some to total X- and Y-bearing sperm, but the differences are slight.)

We can calculate the values of α, i.e. the mean number of primary breaks per sperm per 1000 r., in Y-bearing sperm by making use of data on the relative frequency of breaks in the X-chromosome, the Y-chromosome, and the autosomes given by Bauer *et al.* (1938). We can calculate the value of α in X^{c2}-bearing sperm from consideration of the fact that the X^{c2}-chromosome is 25% longer than the normal rod-X-chromosome. In this way we arrive at the values of α given in Table 5. α has the value 0·75 (cp. p. 17) in the rod-X-bearing sperm, is slightly greater in the X^{c2}-bearing sperm, and slightly less

in the Y-bearing sperm. In the same table s is the proportion of the total number of breaks which occur in the sex chromosome in the three types of sperm. The values of s are given by Bauer *et al.* (1938) for X-bearing and Y-bearing sperm, and are calculated thence for the X^{c2}-bearing sperm by making allowance for the 25 % extra length.

The proportion of rod-X-bearing sperm which after dose D give viable zygotes is given by equation (2) and Table 5 as

$$e^{-\alpha D} S_2, \text{ with } \alpha = 0{\cdot}75 \text{ per } 1000 \text{ r., and } q = 0{\cdot}76. \tag{7}$$

The proportion of Y-bearing sperm which after dose D give viable zygotes is similarly

$$e^{-\alpha D} S_2, \text{ with } \alpha = 0{\cdot}718 \text{ per } 1000 \text{ r., and } q = 0{\cdot}76. \tag{8}$$

Table 5. *Data for sex-ratio calculation*

Type of sperm	α	s	p	q	Formula applicable	Sex-ratio ♀♀ ÷ ♂♂
Y	0·718	0·168	0·24	0·76	(8)	—
X	0·750	0·204	0·24	0·76	(7)	(7) ÷ (8)
X^{c2}	0·788	0·242	0·24	0·76	(10)	(10) ÷ (8)

The calculation for X^{c2}-bearing sperm is a little more complicated. The proportion of X^{c2}-bearing sperm which have r breaks, all of which join, is $e^{-\alpha D}(\alpha q D)^r / r!$. In some of these sperm all the breaks will be in the autosomes, in the remainder one break will be in the X^{c2}-chromosome. (In accordance with the simplification adopted throughout, we do not contemplate the possibility of more than one break occurring in the X^{c2}-chromosome.) Thus, since s is the proportion of breaks which occur in the sex chromosome, we write rs as (approximately) the proportion of sperm in which there is a break primarily produced in the X^{c2} sperm. $1-rs$ is the proportion of sperm in which no break is produced in the X^{c2}-chromosome. For the calculation of the proportion of sperm of the latter class which are viable the formulae used on p. 16 apply, leading to $2^r (r!)^2/(2r)!$. A sperm of the former class, having one break in the X^{c2}-chromosome and $r-1$ breaks in the autosomes, will only be viable when the following conditions are satisfied. The break in the X^{c2}-chromosome must restitute in preference to interchanging with another break (the probability of restituting is $1/(2r-1)$, since a broken end has $2r-1$ broken ends with which joining is possible). It must restitute in the way leading to a viable chromosome (probability $\frac{1}{2}$). Finally, the $r-1$ autosomal breaks must join in a viable fashion, the probability of which is $\dfrac{2^{r-1}[(r-1)!]^2}{(2r-2)!}$. We finally obtain for the contribution to viable sperm provided by sperm with r breaks the expression

$$e^{-\alpha D}\left\{\frac{(2\alpha q D)^r\, r!\,(1-rs)}{(2r)!} + \frac{(\alpha q D)^r\,[(r-1)!]^2\, 2^{r-1}\, rs}{r!\,(2r-2)!\,2\,(2r-1)}\right\}. \tag{9}$$

Simplifying, and summing the infinite series of which this is a term, we obtain for the proportion of X^{c2}-bearing sperm which are viable after dose D

$$e^{-\alpha D}\{-\tfrac{1}{2}s + (1+\tfrac{1}{2}s)\, S_2 - sS_3\}, \tag{10}$$

with $\alpha = 0{\cdot}788$ per 1000 r., $s = 0{\cdot}242$, and $q = 0{\cdot}76$.

Formulae (7), (8) and (10) may be evaluated numerically with the appropriate values of α, s, p, and q, which are collected in Table 5, and the values of S_2 and S_3 tabulated in Table 3. The expected ratio of females to males in the progeny of irradiated X/Y males is evidently (7) ÷ (8), while the ratio in the progeny of irradiated X^{c2}/Y males is (10) ÷ (8). In this way the theoretical curves of Fig. 3, which show the sex ratio as a function of dose, have been computed.

It is seen that the theoretical curves are in fairly good agreement with the experimental data also shown in Fig. 3. The small but systematic deviation between Bauer's experimental results and the theoretical curve for the X^{c2}/Y males can in the main be accounted for by the fact that in addition to the principal causes (*a*) and (*b*) (p. 19) contributing to the distortion of the sex ratio, there are some smaller factors acting in the same direction

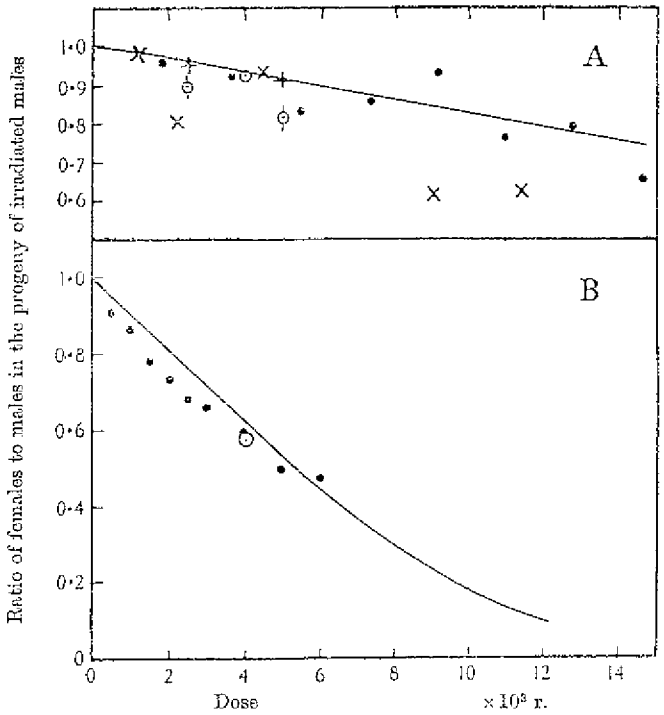

Fig. 3. Depression of sex-ratio in progeny of irradiated males. Curves theoretical; points experiments of: A, X/Y males (+ Hanson (1928), ⊙ Muller (1928), ● Gowen & Gay (1933), ⊙ Bauer (1939*a*), × Catcheside & Lea (1945*a*)). *B*, X^{c2}/Y males (● Bauer (1939*a*), ⊙ Catcheside & Lea (1945*b*)).

which we have not taken into account. One of these is the fact that nearly all of the deletions, and half of the inversions, in the X^{c2}-chromosome will behave as dominant lethals, and these aberrations are not taken into account by our theory which does not allow of more than one break per chromosome. Secondly, a small proportion of breaks of the X^{c2}-chromosome lead to viable losses of the chromosome (Pontecorvo, 1941) and count twice in the sex-ratio distortion, since they lead not only to the disappearance of a female but also to the appearance of a male (XO male). There is thus explanation for the departure between theory and experiment, which is in any event slight.

It is important to notice that the agreement between theory and experiment in Fig. 3 is obtained without there being any arbitrary constants involved in the theory which have to be determined by appeal to the sex-ratio experiments, since α and q were determined already in § 3 by appeal to other experiments.

5. Discussion

The theory of dominant lethals and chromosome aberrations we have developed agrees well with the experimental data, as illustrated in Figs. 1 and 2. The theory involves two

arbitrary constants, α and q, the values of which have to be chosen by reference to the experiments which the theory is fitting. There is always the fear attaching to a mathematical theory involving arbitrary constants that an incorrect theory may be made to fit the experiments owing to the flexibility afforded by the arbitrary constants. Here two confirmations are possible which we think make the fear groundless in the present instance. In the first place we were able to extend the theory to cover the calculation of the distortion of the sex ratio in the progeny of irradiated males. This extension did not involve any fresh arbitrary constants, and the theory satisfactorily fitted the experimental data, as illustrated in Fig. 3. In the second place, we have in a separate paper (Catcheside & Lea, 1945*b*) shown that an analysis of the relative degrees of sex-ratio distortion in the progeny of X/Y and X^{c2}/Y irradiated males leads directly to an approximate estimate of q. The value obtained, $q=0{\cdot}74$, was in good agreement with the value $q=0{\cdot}76$ obtained in the present paper. For these reasons we believe that our dominant lethal theory is not merely a mathematical exercise, but is essentially correct.

As regards the recessive lethal theory, this is admittedly more speculative. That it is consistent with the dominant lethal theory is shown by the following consideration. The analysis of the dominant lethals and chromosome aberrations led to an estimate (p. 19) of 0·199 for the number of primary breaks per sperm produced by 3000 r. in the euchromatin of the *X*-chromosome (in sperm which remain viable). Our analysis of recessive lethals on the basis that recessives are rejoined breaks led independently to an estimate of this same quantity, the figure obtained (p. 14) being 0·226. The agreement between 0·199 and 0·226 is satisfactory.

The dominant lethal theory contains a number of approximations and simplifications inevitable when it is attempted to develop a mathematical theory of a biological process. Some of these we now discuss.

(*a*) The assumption of random rejoining is obviously only an approximation to the truth. It seems fairly certain, nevertheless, that the contrast between the postulate of random rejoining which we have used in the present paper with the postulate that union can only occur between breaks formed within a short distance apart, which we used (Lea & Catcheside, 1942) in discussing interchanges in *Tradescantia* pollen grains, reflects a real difference between the conditions obtaining in the two cases.

(*b*) Having postulated that q is the probability that a given break shall join, it is not certain that we are justified in inferring that q^r is the probability that r breaks shall all join. In other words, it is not certain that q is independent of the dose. The deduction is valid if it is something in the nature of the breakage process which decides whether a break is joinable with other breaks or not. It is only an approximation, and perhaps not a very good one, if it is the chance meeting of broken ends before sister-union occurs which determines whether interchange occurs or whether sister-union, leading to a dominant lethal effect, takes place.

(*c*) The assumption that every break in a nucleus occurs in a different chromosome arm will be in error for doses at which large numbers of breaks are formed.

Errors introduced by (*b*) and (*c*) will be more marked at high doses, and we would not have been surprised therefore had we found departures between theory and experiment at high doses.

The close connexion postulated for the origin of dominant lethals, recessive lethals and chromosome aberrations seems at first sight to be contradicted by the experimental result

(Dempster, 1941) that neutrons and X-rays show a ratio of efficiency which is different for each of the three classes. More extensive experiments of this sort are urgently required. If the experimental result is established, the explanation may lie in q being lower for a break produced by a densely ionizing proton than by an electron. We have obtained some results in our *Tradescantia* experiments (Catcheside & Lea, 1943) which can be interpreted on the basis that a densely ionizing particle makes a less readily joinable break than does an electron, presumably because it does more damage to the chromosome.

As the number of breaks per sperm increases, the probability that a sperm shall be non-viable increases at a disproportionate rate. In consequence the mean number of breaks per *viable* sperm increases rather less rapidly than the first power of the dose. The figures in Table 4 increase approximately as $(\text{dose})^{0\cdot84}$. On the view regarding recessive lethals put forward in § 2, the number of recessive lethals per sperm observed should similarly increase less rapidly than the first power of the dose. $(\text{Dose})^{0\cdot84}$ is not a very marked deviation from linearity, but in view of the fact that the experimental data quoted in Table 1 rather exactly fit a linear law, and are based on a very large number of lethals, the χ^2 test shows them to be significantly at variance with a $(\text{dose})^{0\cdot84}$ variation, and they cannot be fitted satisfactorily by any power of the dose lower than $(\text{dose})^{0\cdot95}$. (Fitting the experimental data to this formula makes $\chi^2 = 5\cdot9$, $n=2$, $P=0\cdot05$.)

We are not disposed to regard this disagreement as necessarily fatal to the point of view regarding recessive lethals which we have put forward. All the approximations made in the development of the theory have erred in a direction likely to exaggerate this departure from linearity. That the mean number of primary breaks per viable sperm increases less rapidly than the first power of the dose is due to the probability that a sperm shall be viable, decreasing rapidly with increase of the number of breaks primarily produced in it, and the departure from linearity will be exaggerated if the calculation exaggerates this decrease of probability with increase of the number of breaks. Our expression for the probability of a sperm being viable in which r primary breaks are produced was

$$\frac{q^r\, 2^r\, (r\,!)^2}{(2r)\,!}. \tag{11}$$

All the approximations introduced into the calculation act in a direction to make this estimate of the probability too low. Fano (1943) gives an expression for the probability of a sperm being viable which has r primary breaks in l chromosome arms, $(r \geqslant l)$, and it is greater than (11) by a factor which is approximately 2^{r-l}. Thus our neglect of the possibility that more than one break may occur in the same chromosome arm leads to underestimation of the probability of the sperm with r breaks being viable. In the second place (11), and also Fano's expression, were calculated on the basis of random joining of broken ends. Any departure from randomness will be in the direction of restitution being preferred to illegitimate union, thus further making the probability of a viable nucleus higher than expression (11). Thus the power 0·84 in the $(\text{dose})^{0\cdot84}$ formula we have referred to is likely to be too low. Finally, in addition to lethals due to breaks there doubtless are some lethals (though we believe them to constitute a minority) not associated with breaks. Some of these will be point lethals analogous to visible mutations and will be strictly proportional to dose. Others will be position-effect lethals and will increase more rapidly than the first power of dose. The addition of these neglected classes would bring the dose-variation curve still nearer to linearity.

SUMMARY

The suggestion is put forward that radiation-induced recessive lethals, or a large proportion of them, are due to chromosome breaks. About one-third of all the chromosome breaks primarily induced by the radiation are lethals. If the break restitutes, a lethal unaccompanied by chromosomal aberration (type A lethal) results. If the break takes part in chromosome interchange a type C lethal, which is associated with chromosomal structural change, results. Arguments are given against the alternative position-effect explanation of type C lethals.

A quantitative theory of dominant lethals is developed on the basis that the dominant lethals are a mixture of single breaks which fail either to restitute or to interchange but instead undergo sister-union, and of non-viable chromosomal structural changes involving two or more breaks. The experimental curve of variation with dose of the yield of dominant lethals is successfully fitted, and also the curve of the yield of viable structural changes.

It is shown that the recessive lethal and the dominant lethal theories are consistent in that they require the same postulated number of primarily produced chromosome breaks per unit dose (namely, 0·75 breaks per sperm per 1000 r.). Experiments on the distortion of the sex ratio in the progeny of irradiated males with ring-shaped or rod-shaped *X*-chromosomes are also shown to be consistent with the theory.

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