

THE BEARING OF THE FREQUENCIES OF X-RAY INDUCED INTERCHANGES IN MAIZE UPON THE MECHANISM OF THEIR INDUCTION

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Two hypotheses have been developed to account for the induction of structural changes of chromosomes by X-rays and other short-wave radiations. According to one, which may be known as the breakage hypothesis, breaks are induced separately in the chromosomes and rearrangements are produced when the breakage ends subsequently rejoin by chance in new ways (Stadler, 1931 *a, b*, 1932). According to the other, which may be called the contact hypothesis, the rearrangements occur when two or more chromosome regions in propinquity or in contact are affected by the same X-ray hit (Muller, 1932). The latter hypothesis is essentially like that advanced by Serebrovsky (1929), and extended by Dubinin (1930), to explain spontaneous as well as induced structural changes. The underlying distinction between the two hypotheses is that the chromosome breaks, inferred in one structural rearrangement, are produced either by separate hits or by a single hit.

The problem of deciding whether the breaks inferred in a structural change induced by irradiation have a common or separate causative origin may be approached by considering the relative frequencies of different classes of rearrangements. For this purpose, we shall deal in this paper with the frequencies of different kinds of induced interchanges in maize, especially those that involve more than two chromosomes in a nucleus.

MATERIAL AND METHODS

The material consisted of six families of F_1 maize plants, totalling 417 individuals, raised from kernels obtained when pollen treated with X-rays was applied to untreated ears. The dosage given to the pollen was "2000 r.u." (see footnote to Table II). Determinations of the presence of induced interchanges was made by observation of rings of four or more chromosomes at diakinesis and metaphase I in acetocarmine preparations of pollen mother cells.

RESULTS

The data are given in Table I. Of the plants grown, a fair proportion gave no information. Several causes have contributed to this, namely: (1) weak plants that produced no tassel; (2) plants sampled at the wrong stage of development and in which no diakinesis or metaphase I pollen mother cells could be found; and (3) asynaptic (two) and antherless (one) plants. The first and second classes totalled 86 plants out of the 417 raised.

TABLE I

Diakinesis (and metaphase I) arrangements of chromosomes in F₁ maize plants raised from the kernels given by untreated ears fertilized by pollen X-rayed with 2000 r.u.

Diakinesis arrangements of chromosomes	Nos. of plants in families A to F						Total plants
	A	B	C	D	E	F	
10 (2)	46	64	51	23	20	31	235
(4) + 8 (2)	23	22	13	5	10	8	81
(6) + 7 (2)	1	2	0	0	0	1	4
2 (4) + 6 (2)	2	1	1	0	0	1	5
(8) + 6 (2)	0	1	1	0	0	0	2
(6) + (4) + 5 (2)	0	0	0	0	0	1	1
Total plants	72	90	66	28	30	42	328

Stadler & Sprague (1937) obtained similar data for comparison with the frequency of ultra-violet induced interchanges, but it was not reported in detail. They have very kindly allowed me to publish the information upon which their summary was based. It is given in Table II, together with a summary of the new data in Table I.

TABLE II

Relative frequencies of different kinds of X-ray induced interchange in maize, recorded as diakinesis configurations in heterozygotes

Source and dose	10 (2)	(4) + 8 (2)	(6) + 7 (2)	2 (4) + 6 (2)	(8) + 6 (2)	(6) + (4) + 5 (2)	Total plants
Table I, "2000 r."**							
Nos. of plants	235	81	4	5	2	1	328
%	71.7	24.7	1.2	1.5	0.6	0.3	
Stadler & Sprague, 1333 r.							
Nos. of plants	54	31	4	6	1	0	96
%	56.3	32.1	4.2	6.3	1.1	0	

* The dose of "2000 r." is probably under 1000 r.u.; it cannot, at present, be fixed with confidence.

The data show a proportionality between dosage and rate of induced structural change. They also show that while the absolute frequency of

rings of six and of two rings of four chromosomes rises markedly with increased dosage, yet the ratio of their frequencies remains constantly near equality.

DISCUSSION

It will be shown that the maize data summarized in Table II provide evidence for the view that rearrangements of chromosomes are induced by X-rays when the latter affect two, or more than two, chromosome regions in contact or in propinquity. The main facts adduced in support of this contention are (1) the induced interchanges between four chromosomes taken two at a time are rather more frequent than those between three chromosomes, and (2) the ratio of the frequencies of these two classes is a constant at different X-ray doses. The expectations on our two hypotheses have been deduced by the following reasoning. The assumptions and conventions used are the same as those in the previous paper (Catcheside, unpublished).

The breakage hypothesis

The breaks induced in the treated nuclei have a mean frequency proportional to the dose administered. At a given dose, and therefore a given mean frequency of breaks, the latter are distributed at random amongst the treated nuclei. This distribution must be Poisson and therefore the frequencies of nuclei with 0, 1, 2 and more breaks are readily calculable. The breaks in a nucleus may be spread amongst one or more chromosomes in the nucleus; this distribution, if random, may be calculated. Given the presence of breaks in the chromosomes of a nucleus, recoverable structural changes would be produced only if all the breakage ends rejoined, some, or all, of them in new ways.

For the purpose of calculation I have assumed (1) that all the chromosomes have an equal chance of being broken, (2) that the occurrence of two or more breaks in one chromosome may be neglected, (3) that all breakage ends rejoin, (4) that breakage ends rejoin at random. In Table III are given the proportionate contributions to different classes of chromosome reconstruction in the progeny calculated on the above conditions when the nuclei have various numbers of breaks induced in them.

It is clear that the contribution to interchanges showing as two rings of four chromosomes in the heterozygote will always be less than that to interchanges showing as rings of six chromosomes, no matter however frequent are the breakages per nucleus. The discrepancy would decrease

with increasing frequency of mean breaks per nucleus as shown in Table IV. The frequencies in this table are totals for the progeny of all the treated nuclei in each population having different mean numbers of induced breaks. They have been calculated from the values given in Table III and the expected random frequencies of nuclei with 0, 1, 2, 3 or more breaks (cf. Catcheside, previous paper, Table III) when the mean number of breaks per nucleus has different values. The contributions have been summated for each distinguishable class that is recoverable

TABLE III

Breaks per nucleus	Proportionate contributions to various classes of progeny							
	Normal	Invi- able inter- change	Viable interchange					
			(4)	(6)	2 (4)	(8)	(6) + (4)	(10)
0	1							
1	1							
2	1/3	1/3	1/3					
3	1/15	9/15	3/15	2/15				
4	1/105	81/105	6/105	8/105	3/105	6/105		
5	1/945	825/945	10/945	20/945	15/945	30/945	20/945	24/945

TABLE IV

Theoretical percentages per 100 gametes rayed of normal and different kinds of interchange recovered in the progeny. The interchanges are recorded by the configurations given in heterozygotes with the normal

Mean nos. of breaks per nucleus	Normal	(4)	(6)	2 (4)	(8)	(6) + (4)	(10)
0.1	99.67	0.17	—	—	—	—	—
0.2	98.83	0.55	0.01	—	—	—	—
0.4	95.65	1.95	0.10	—	—	—	—
0.8	85.6	5.61	0.57	0.02	0.05	—	—
1.6	61.8	12.0	2.34	0.18	0.36	0.04	0.05
3.2	25.7	12.5	3.55	0.68	1.37	0.25	0.3
6.4	2.9	3.8	2.6	0.83	1.35	0.6	0.65

in the progeny. The ratio of induced rings of six chromosomes to cases of two rings of four chromosomes each is high at low mean frequencies of breaks per nucleus and therefore at low doses. It diminishes with increasing breakage frequency (and higher doses), approaching to a value of approximately three to one.

Clearly, if the chromosome breaks are independent of one another in their origin, interchanges involving three chromosomes should be considerably more frequent, especially at low doses of X-ray, than those involving four chromosomes two at a time. Further the ratio of the former to the latter (cf. Table IV) should decrease with increasing

dosage. Neither of these expectations are fulfilled by our observations (Table II). Moreover, rings of eight chromosomes should be twice as common as two rings of four chromosomes, but they are not.

Taking into account the possible factors which have been ignored so far, namely cases of two or more breaks in one chromosome, failure of reattachment and more frequent reattachment of a broken end to its original position, the ratios between rings of six chromosome plants and two rings of four chromosome plants implied by Table IV are minimal. For, two rings of four chromosomes always require one more break for their production than do rings of six chromosomes and therefore they are at a greater disadvantage from causes leading to discrimination.

The contact hypothesis

On this hypothesis, cases of interchanges between four chromosomes two at a time and showing as two rings of four in the heterozygote may have resulted from (1) one hit affecting four chromosomes in contact at one point, or (2) two hits separately affecting two pairs of chromosomes in different parts of the same nucleus. The cases of interchange between three chromosomes, showing as rings of six in the heterozygote may have come from (1) one hit affecting three chromosomes in contact at one point, or (2) from two hits separately affecting two pairs of chromosomes having one member in common. We know that single hits rarely affect three or more chromosomes at once so that the first possibility in each case may be neglected. Hence, the problem of finding the expected ratio of rings of six chromosomes to two rings of four chromosomes is simply that of determining the relative frequency with which a second interchange would and would not involve a chromosome already interchanged with another chromosome.

There are forty-five ways in which the second interchange may occur in a maize nucleus with ten chromosomes. Of these one would be a further interchange between the two chromosomes already subjected to interchange. Sixteen would be an interchange between one of the two chromosomes in the first interchange and one of the eight not so involved. Finally, twenty-eight would be an interchange between two of the eight chromosomes not already interchanged. Hence, provided the occurrence of the second interchange is independent of the first, the expected ratio of plants with rings of six chromosomes to those with two rings of four chromosomes should be 4 : 7. Further it should be independent of the frequency of the causes leading to interchange and therefore independent of the dosage.

CONCLUSIONS

The observations fit the statistical requirements of the contact hypothesis. They do not fit the requirements of the breakage hypothesis if it be assumed, as in our calculations, that all breakage ends rejoin at random. But we must determine whether any modification of the latter postulate would admit the observed results.

Prof. L. J. Stadler (*in litt.*) has pointed out that, if breaks are produced singly by hits and are very numerous and transitory, a high proportion of reattachments would be in the original positions. Of the remainder, a large proportion would be interchanges (or inversions) involving broken ends which were within a given limited distance at the time of breakage. The rest of the reattachments would involve broken ends relatively far apart at the time of their production.

On the single hit-single break hypothesis, as shown above, progressive translocations involving three breaks should be more frequent than double translocations involving four breaks. But if reattachment of nearby ends were favoured, the frequency of progressive translocations would be correspondingly lowered. With three breaks distributed at random, the two which were closest together would tend to interchange, leaving the third to revert; with four breaks, the interchanges could often be two and two. The small percentage of reattachments of ends not near together could produce some progressive translocations but their number would depend upon the extent of the spatial selective tendency. Consequently the cases observed as rings of six chromosomes, in the F_1 heterozygotes, may be mostly double translocations with one chromosome in common, progressive translocations being relatively few in number. If so, their frequency would not be much more than four-sevenths that of cases of two rings of four chromosomes, and it would increase with dosage to about the same extent as the latter class of heterozygote.

Workable mechanisms for the production of structural changes by X-rays are therefore: (1) a contact mechanism, in which chromosome regions close together or in actual contact are together affected by one hit; (2) a breakage mechanism, in which the breakages are relatively transitory and in which translocations are produced chiefly following breakage of chromosomes in nearby positions. The biological difference between them is not very great and is perhaps merely academic. Further, they are not necessarily mutually exclusive. Physically, the difference lies in whether one, or more than one, hit is involved in the production of a single structural rearrangement.

In the case of irradiated *Drosophila* sperm about 12% of the induced rearrangements are progressive, that is involving three chromosome regions together (Catcheside, unpublished). If the contact hypothesis were the correct one, such a high frequency of progressive rearrangements effected by the radiation could be attained only if all chromosome regions were in contact with, or in very close proximity to, one or more other chromosome regions in the treated nuclei. This would be realized if the chromosomes filled most of the nuclear space, except that occupied by the nucleolus; indeed this appears to be the case when resting nuclei are examined. It is doubtful, however, whether very complex progressive contacts involving as many as nine or ten chromosome regions (Dubinin & Khvostova, 1935) could be reasonably expected.

With the uncoiling of chromosomes at telophase, the formation of relic spirals and the subsequent hydration and swelling of the chromosomes in the formation of the resting nucleus, parts of the same chromosome are likely to be brought close together relatively more frequently than are parts of different chromosomes. It follows, on either hypothesis that inversions should be relatively more frequent than interchanges. It was found that X-raying *Drosophila* sperm produced inversions twice as frequently as they should be if translocations between different parts of the same chromosome arm were relatively no more frequent than between different chromosome arms.

The mechanism of structural change through disturbance of two or more chromosome regions in propinquity evidently determines the production of reciprocal changes. We have no unequivocal evidence to show us whether temporary or permanent single breaks can be induced. However, cases of terminal deficiency (Demerec & Hoover, 1936) and the recovery phenomenon in maize endosperm (Stadler, 1930) are perhaps most simply explained on that basis. Unreported observations of the present author on irradiated ring X-chromosomes in *Drosophila* show a total absence of simple breaks in the ring chromosome. But we cannot tell whether to interpret this as a non-occurrence of them or as obligate refusion at a relatively early stage in the ontogeny of the animal.

SUMMARY

A study of X-ray induced interchanges in maize has shown, in the F_1 progeny from irradiated pollen, that individuals with two rings of four chromosomes are slightly more frequent than those with a ring of six chromosomes. Similar relations between these two classes hold at different X-ray doses. These facts may be accounted for on the contact

hypothesis or on the breakage hypothesis if it be supposed that structural rearrangements chiefly follow breaks occurring in nearby positions. The data cannot be used to decide whether translocations require one or more hits for their production.

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