PARTIAL BREAKAGE OF SALIVARY GLAND CHROMOSOMES

B. M. SLIZYNSKI

Institute of Animal Genetics, Edinburgh University, Edinburgh, Scotland

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IN 1936 MARSHAK published a report of his experiments with very high dosage X-ray radiation of Drosophila. He treated six to eight hours old embryos with 5,000 r units and found that most of the salivary gland chromosomes showed one or more translocations. No details are given in regard to the diameter of the parts of the chromosomes involved in translocations and there is no mention of inversions or deficiencies in this series of MARSHAK's experiments. Embryos of the 10–12 hour stage treated with 5,000 r units showed deficiencies in the chromatic bands, half a band being missing in one of the homologues. In the last series of MARSHAK's experiments three day old larvae treated with 20,000 r units showed no detectable effects in their salivary gland chromosomes.

The present report deals with the effects of small dosage X-ray radiation on young embryos of *Drosophila melanogaster* and contains data from six experiments. Experiments with some other mutation producing agents show similar results.

MATERIAL AND METHODS

Flies of the Florida-4 stock were used throughout, the cultures being kept at 22°C. The first experiment was carried out in the following way: about 50 virgin females one to three days old were mated to males from the same culture bottle collected at the same time. The flies were put together at 10 P.M. and were removed from the bottle at 6 A.M. the following day. All eggs, numbering about 80, which were laid during this period were irradiated at the same time *i.e.* at 10 A.M., with a dosage of 500 r units. It may be accepted that the oldest embryos were about 12 hours old and the youngest about four hours old at the time of treatment. After irradiation the eggs were transferred to fresh food and kept at 22°C for two days; afterwards they remained at room temperature. Fully grown larvae were dissected and slides were made using the acetocarmine squash method and mounting in Euparal; some preparations were also stained by means of the Feulgen reagent. The slides were given consecutive numbers and their order thus marked approximately the age of the embryo. This method of timing, although not an exact one, was found to be very convenient in experiments concerned not with the exact age of the embryo but, for instance, with the optimum dosage of r units required to produce changes. In other experiments the ordinary method of timing was used.

Microscopical observations were made with a 1.4 NA 90× oil immersion

objective, and drawings with the aid of camera lucida and 30× eyepiece, the details being drawn in with 7× eyepieces. Microphotographs were made by MR. G. R. KNIGHT to whom the author owes gratitude.

The main difficulty in recording chromosome breaks is caused by the fact that each structural change occurs only once. This, in conjunction with the fact that even on the best slides only a few nuclei are completely analyzable is a serious disadvantage of the method when employed for the study of the frequency and distribution of chromosome breaks.

RESULTS

Structural Changes in the Salivary Gland Chromosomes

Generally speaking the results of the treatment include all known types of structural change—but the changes met with in these experiments differ fundamentally from other cases so far described, in that they do not involve the whole width of the chromosome. For instance deficiencies were observed which did not remove a complete section of the chromosome but only a longitudinal part of it so that it is still capable of pairing with its homologue along its whole length. In such cases where a piece between the points of breakage is missing the two broken ends rejoin. Since however, the elimination shortens the affected region of the chromosome a peculiar "archer's bow" structure is obtained in which the main body of a chromosome corresponds to the elastic wooden part of a bow while the affected part is stretched and may be compared to the string. Such side connections produced by rejoining of the ends are usually very thin; their diameter can best be estimated at their point of separation from the main body. Changes of this type induced in salivary gland chromosomes are called partial structural changes since they affect only a part of a chromosome. Many partial structural changes may appear in mutual combination on a single chromosome or they may involve more than one chromosome. Sometimes very complicated figures were seen where analysis was impossible.

Typical partial structural changes are illustrated in figure 1, which contains camera lucida drawings and their probable interpretation; in the latter, for the sake of simplicity, the number of chromonemata has been limited to two in a univalent. In addition there are microphotographs of parts A and C.

Drawing A shows a partial deficiency of medium size in divisions 4 and 5 (BRIDGES' reference system) of the X chromosome.

Drawing B shows a partial inversion at the free end of the right arm of chromosome 3. There is no detectable partial deficiency connected with the inversion.

Drawing C shows a partial translocation between the X chromosome and the left arm of chromosome 2 in combination with the loss of both centromeres involved.

Drawing D shows a partial deficiency in the left arm of chromosome 2; the side connection unites the beginning of division 39 with a chromocentral mass. It is not possible to say to which chromosome this part of the chromocentral mass belongs.

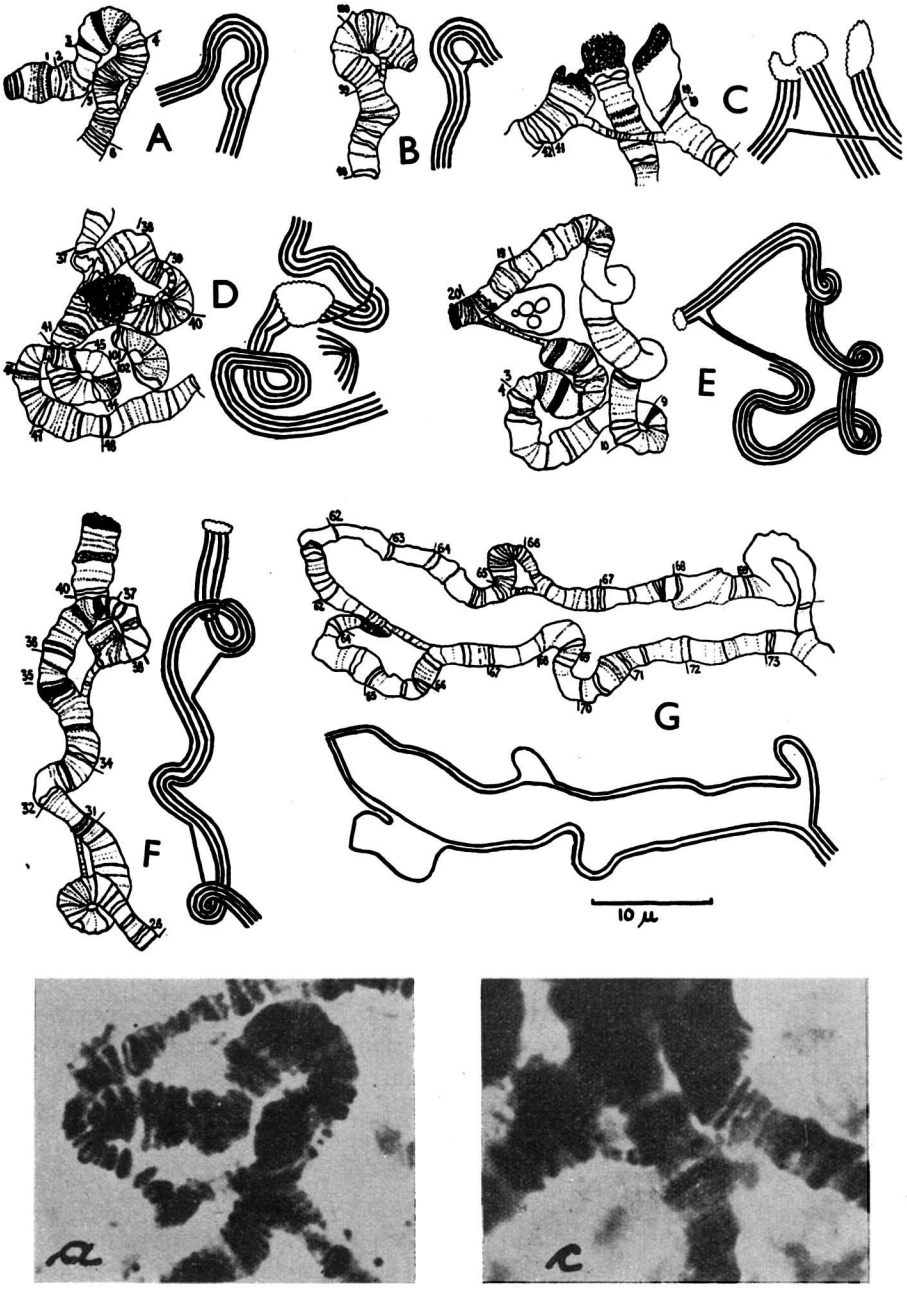

FIGURE 1.—Examples of partial structural changes. Camera lucida drawings and diagrammatic interpretations: A—deficiency, B—inversion, C—translocation, D—deficiency, E—ring formation, F—deficiency in combination in inversion, G—two deficiencies each in one of the homologues; a—microphotograph of A, c—microphotograph of C.

Drawing E shows the free end of chromosome X joined by a thin side connection to the base of the same chromosome at division 20. At the point where it joins the base, its greatly extended subdivision 1A is split into two arms; one goes over into the proximal part of the base while the other turns distally. Two ring chromosomes have thus been produced, the smaller of them being acentric.

Drawing F shows two side connections in the left arm of chromosome 2. One of them joins the body of the chromosome at divisions 38 and 35, and the other at 31 and 28. The first side connection involves a partial deficiency in combination with partial inversion, the other represents simply a partial deficiency.

Drawing G shows two largely non-paired univalents (the left arm of chromosome 3) each having a partial deficiency. One point of breakage, namely that in division 66 is common to both partial deficiencies.

It has been noticed in all the experiments that the older the embryos were at the time of treatment the thinner were the side connections. The nature of the data, however, would not justify any statistical treatment of this correlation. There are difficulties in the exact measuring of the diameters which are as a rule very much affected by the stress of extension. Those produced in one 13 hours old embryo were estimated to involve 1/16th part of the chromosome, which is however at the limit of optical visibility. The side connections observed in chromosomes belonging to cells from various parts of the same salivary gland may show a certain amount of variation in width which is probably due to the different age of the chromosomes at the time of treatment. In one of the experiments in which embryos were treated with 400 r units when 10–13 hours of age there were among 143 well determined structural changes 79 (55 percent) deficiencies, 24 (17 percent) inversions and 40 (28 percent) translocations.

Distribution of Chromosome Breaks

The X-raying of salivary gland chromosomes in Drosophila can with some limitations be used for studying chromosome breaks. No special experiments were carried out on the frequency distribution and dosage relation of partial structural changes, but during the experiments some data were collected on the subject.

The most extensive study on the distribution of chromosome breaks in all chromosomes is that of BAUER, DEMEREC and KAUFMANN (1938) who examined salivary gland chromosomes of 1,765 larvae whose male parents were treated with X-rays. They obtained 1,038 breaks. In 1939 BAUER published another paper on chromosome breaks obtaining similar results at least so far as the problems discussed here are concerned.

In one of the experiments reported here one larva which was treated with 600 r units as a 10–13 hours old embryo yielded 192 chromosome breaks. Their distribution is represented in figure 2, where it is compared with data obtained by BAUER, DEMEREC and KAUFMANN. Although the frequencies

are much higher for their material the two curves have much in common.

In figure 3, the distance between two related breaks is compared with that obtained by the same authors and also shows good agreement between the two sets of data. The low frequency at the left end of both curves, corresponding to the distances smaller than one division is probably due in both cases to the difficulties of detection and determination of such small structural changes.

The results of the present paper show that a few threads belonging to the same bundle can be broken without the destruction of the remainder. Thus it can be concluded that the action of X-rays is limited to a very small field and that the spreading of the effect of radiation does not necessarily transect the whole of the chromosome.

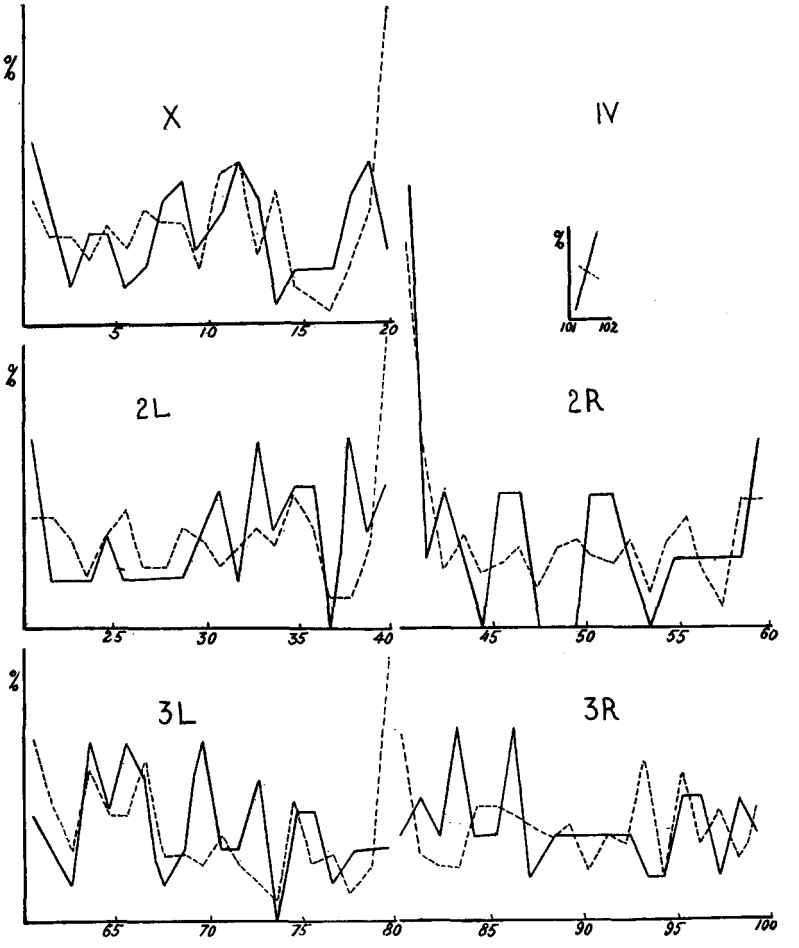

FIGURE 2.—Frequency distribution of chromosome breaks. *Solid line:* data from one larva treated with 600 r units as a 10–13 hours old embryo. *Broken line:* data from BAUER, DEMEREC and KAUFMANN (1938) from 1,765 larvae, whose male parents were treated with X-rays. *Ordinate:* frequency in percentage for each chromosome limb. *Abscissa:* BRIDGES' divisions of cytological map.

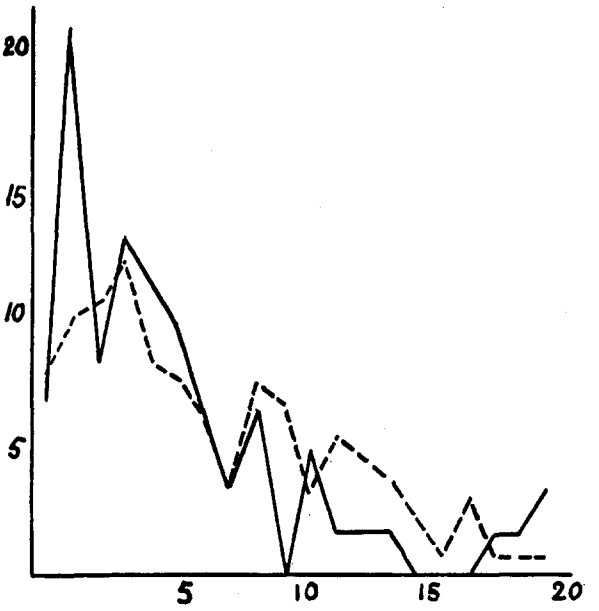

FIGURE 3.—Comparison of distances between two related breaks in the chromosomes. Data from BAUER, DEMEREC and KAUFMANN are represented by broken line, those from the present experiment by continuous line. *Abscissa:* distance in BRIDGES' divisions of salivary gland chromosomes. *Ordinate:* frequency.

DISCUSSION

There are at present three main theories of the structure of salivary gland chromosomes of Dipteran larvae.

According to the first theory in each giant chromosome there is one single thread. During the larval development there is a considerable increase in its size accompanied by coiling. Parts of these coils become dissolved away thus giving the appearance of interband spaces separating the individual bands *i.e.* those parts of the coil which did not undergo dissolution (ALVERDES 1912; KOSSWIG and SENGÜN 1947).

The second theory of their structure assumes that in a univalent chromosome there are only two chromonomata which are either directly visible or may be made visible by appropriate technique. The larger diameter of giant chromosomes is due to an accessory material which may appear in form of numerous secondary chromonemata (CALVIN, KODANI and GOLDSCHMIDT 1940, and KODANI 1942.)

The third theory is the polytene theory which postulates that the giant chromosomes are composed of a large number of threads. These are homologous and mutually equivalent *i.e.* all of the same rank. They are either at the limit of optical visibility or entirely submicroscopic. A giant chromosome is produced by repeated internal divisions of the original chromosome but there is no certainty about how and when these divisions take place (KOLTZOFF 1934; BRIDGES 1935; METZ 1935; BAUER 1935).

The results of the present paper are distinctly in favor of the polytene theory and in this way they support recent studies by GLANCY (1946) who using a refined micrurgical technique was able to demonstrate directly the polytene nature of giant chromosomes by successful isolation of a single fibril. One objection which might have been raised against her evidence is that isolation of fibrils was made in a chromosome which might have been already subject to some changes. The evidence from the present paper disposes of this objection since it shows that salivary gland chromosome can be partitioned *in vivo*.

The data collected in the present paper may partly help in determining the time at which the reproduction of the chromonemata sets in. From the evidence concerned with the thickness of side connections and its correlation with the age of the embryo at the time of treatment it may be concluded that the internal reproduction starts shortly after the end of mitotic divisions.

On the other hand it may be suggested that the relative thickness of side connections does not necessarily prove reproduction of chromonemata. The reasoning is as follows: the same number of strands is present in younger and in older chromosomes but the chromonemata of the old chromosomes are further separated laterally and besides that grow in thickness. In younger chromosomes when the chromonemata are close together several of them might be broken by one hit while in older ones where they are more apart only single chromonemata may be broken, and this would also give the results observed.

The objection is based on the assumption that in mitotic chromosomes of last anaphase the number of threads in each chromosome is the same as in the mature salivary gland chromosomes.

However, BAUER (1935) has shown in Chironomus that salivary gland chromosomes consist of threads in a considerable number which is much higher than that ever assumed for mitotic chromosomes.

And since the side connections described in the present paper may be less than 1/16th of the diameter of a chromosome the number of threads present at the last mitotic division would have to be at least 16 if not much more. Similar evidence is supplied by GLANCY'S data (1946).

Two estimations made by MULLER (1935) give the average diameter of a single chromonema as 0.012 microns (one estimate—0.02, the other—0.004). The diameter of a univalent salivary gland chromosome is about 3 microns *i.e.* about 250 times this figure. Therefore a single chromonema would have to increase hundreds of times in thickness to attain the size of a fully matured salivary gland chromosome. If there were two, four or even eight chromonemata in the original chromosome the rate of their postulated increase would still have to be very high.

On the other hand it could be that the chromonemata do not grow but that the insertion of accessory material between the chromonemata is responsible for the final diameter of salivary gland chromosomes. In such a case one would expect to detect these accessory materials even optically since their amount would have to be very large.

The large diameter of salivary gland chromosomes lends itself more readily

to explanation on a theory of internal multiplication than by such enormous growth rates in individual chromonemata or the insertion of such large amounts of accessory material. JACOBJ'S data, quoted by MULLER (1941), strongly suggest that there is a periodic increase in the number of elements during the growth of a nucleus in salivary glands.

If the number of threads both in mitotic and in salivary gland chromosomes is constant it may be either small or large. A large number does not agree with recent ideas of the structure of mitotic chromosomes while the small number does not agree with polytene structure of salivary gland chromosomes. It may be therefore safely inferred that the number of threads is increasing during the development of salivary glands.

The question of internal multiplication of salivary gland chromosomes can not be directly and finally solved on the evidence presented here. Perhaps a new experiment with two successive treatments separated by a short time interval could produce a partial structural change in the side connections themselves thus proving beyond doubt that these consist of minor threads which increase in number during the development.

SUMMARY

Low dosage X-ray treatment (400–600 r units) of *Drosophila melanogaster* embryos when applied in early developmental stages produces in the salivary gland chromosomes partial structural changes affecting only a few of the chromonemata of which the chromosome is composed.

Partial structural changes include deficiencies, inversions, translocations and ring chromosomes; partial structural changes may also appear in mutual combination.

Structural changes in which a few fibrils are thus separated *in vivo* from the remaining mass of the chromonemata form confirmatory experimental evi-evidence of the polytene nature of salivary gland chromosomes of Diptera.

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