FURTHER STUDIES ON SENSITIVITY OF CHROMOSOMES TO IRRADIATION AT DIFFERENT MEIOTIC STAGES IN OÖCYTES OF SCIARA[1]

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INTRODUCTION

NUMEROUS investigators have presented evidence indicating that the sensitivity of chromosomes to short wave irradiation may differ at different stages in the cell cycle (see below for references), but thus far we have no clear idea of the detailed nature or the cause of these differences in terms of the mechanisms involved. The present investigation represents an attempt to secure additional specific information on the subject in material which is in certain respects particularly favorable for the purpose. The organism used is *Sciara ocellaris* Comst. Earlier studies (METZ and BOCHE 1939; METZ and BOZEMAN 1940, 1942; REYNOLDS 1941; BOZEMAN 1943) have shown that in the maturing oöcytes of this species a very wide sensitivity range is found when different stages are compared. The main criteria used are the frequency and the nature of chromosome rearrangements induced at different stages of the meiotic cycle. In the present work these earlier studies have been extended, with especial regard to the relation between the variation in sensitivity and the developmental changes in behavior and physical characteristics of the chromosomes. Owing to the fact that in Sciara the oöcytes all develop synchronously, and to the fact that the genus belongs to the Diptera, it is possible to identify the meiotic stage and chromosome condition at the time of irradiation and also to make use of the giant salivary gland chromosomes for detailed analysis of the nature of the recovered rearrangements. So far as we are aware, no other material combining these advantages has been used for the present purpose.

An abstract covering most of the present work was published in 1943 (BOZEMAN 1943). Unfortunately, the study was interrupted at that time by war activities and there has been no opportunity to complete it. Likewise, completion of the present manuscript has been long delayed. The interpretations were discussed and some of them presented in abstract form in 1947 (METZ 1947). Since a few points remain to be cleared up, full discussion will not be attempted here; but the data on hand will be presented in some detail so as to be available for future reference. Especial attention will be given here to three points: (1) the possible significance of the great change in apparent sensitivity of the chromosomes which occurs almost abruptly at the time of onset of the first oöcyte division, (2) the unusual phenomenon of apparently

[1] The present study has been carried on with the aid of grants, at different times, from the CARNEGIE INSTITUTION OF WASHINGTON, the AMERICAN PHILOSOPHICAL SOCIETY and the ROCKEFELLER FOUNDATION.

complete insensitivity which precedes this change and (3) the apparent correlation between type of rearrangement induced and stage treated—that is, differences in relative frequencies at different stages.

In considering the literature on variations in sensitivity to irradiation it is difficult to determine how much is pertinent to the present topic. Many studies which do not consider mitotic stages or chromosome rearrangements as such may nevertheless have a bearing on our general problem. Consequently the following brief resumé includes studies of several kinds. The results are summarized in table 1 below. General reviews are given by PACKARD (1931), OLIVER (1934), GOODSPEED and UBER (1939) and SPARROW (1944). The differences investigated include sex differences, age differences, variation in mitotic and meiotic stage, variation in external conditions and variation in kind, dosage and intensity of irradiation. From the results of such investigations deductions have been made as to the method of action of the irradiation and as to the response of the cell to irradiation, but the full significance is not clear in any one case.

TABLE 1

Results of earlier studies on variation in sensitivity to irradiaiton

AUTHOR	CRITERION OF SENSITIVITY	MOST SENSITIVE STAGE
Mottram 1931	Reduction of growth	Mitotic stages
Henshaw and Francis 1935	Retardation of growth	Premitotic
Guyer and Claus 1939	Growth of carcinomata	Colchicine metaphases
Mottram 1931	Hatching of eggs	Metaphase and anaphase
Whiting, A. R. 1940	Hatching of eggs	Metaphase and anaphase
Sonnenblick 1940	Hatching of eggs	Mature eggs (metaphase or anaphase?)
Demerec and Kaufmann 1941	Dominant lethals in sperm	Mature sperm (resting stage)*
Demerec, Kaufmann, Sutton and Fano 1941	Dominant lethals in sperm	Mature sperm (resting stage)
Strangeways and Oakley 1923	Delay in mitosis	Preprophase†
Strangeways and Hopwood 1926	Delay in mitosis	Preprophase
Spear 1932	Delay in mitosis	Preprophase
Mottram, Scott and Russ 1926	Cell death	Mitotic stages
Henshaw and Cohen 1940	Delay in cleavage	Preprophase
Carlson 1938, 1940, 1942	Stoppage of mitosis	Middle prophase
Stone 1933	Chromosome aberrations	24 hours before division
Marshak 1935, 1937, 1939	Chromosome aberrations	Prophase
Gustafson 1937	Chromosome aberrations	Prophase
Eker 1937	Chromosome aberrations	More condensed chromosomes
Sax 1938	Chromosome aberrations	Meiotic prophase
Sparrow 1943, 1944	Chromosome aberrations	Anaphase and metaphase
Whiting, A. R. 1941	Chromosome aberrations	Metaphase
Rick 1943	Pollen abortion	Meiotic prophase

* Difference illusory, according to authors.

† Possibly purely physical phenomena, that is, viscosity changes.

Various standards have been employed to measure the sensitivity of cells at the different mitotic stages, and the choice of a standard has probably affected the results and their interpretation. The same standards, however, when applied to different organisms yield divergent results. Using growth rate as a measure of sensitivity to irradiation the most sensitive stages were found by MOTTRAM (1931) to be the mitotic stages, by GUYER and CLAUS (1939) to be metaphase, by HENSHAW and FRANCIS (1935) to be the premeiotic period. Using mortality as a standard the most sensitive stages were found by MOTTRAM (1931) and A. R. WHITING (1940) to be metaphase and anaphase. Similarly, SONNENBLICK (1940) found mature (anaphase ?) eggs of Drosophila most sensitive and DEMEREC and KAUFMANN (1941) and DEMEREC, KAUFMANN, SUTTON and FANO (1941) found mature sperm (resting stage ?) most sensitive.

Delay in mitosis or cleavage following irradiation is probably not comparable with chromosome aberrations or with mortality as it is a temporary phenomenon and may be due to a reversible alteration in viscosity of the cytoplasm. Division is generally delayed most when organisms are irradiated during preprophase stages (STRANGEWAYS and OAKLEY 1923; MOTTRAM, SCOTT and RUSS 1926). CARLSON (1938, 1940, 1942), however, found the middle prophase most sensitive.

When we consider the production of chromosomal aberrations by irradiation at various mitotic stages we find considerable confusion. This is brought about by the use of a variety of organisms, by examination at widely varied intervals after treatment (*e.g.*, at the succeeding anaphase in Tradescantia and in the salivary glands of developing F_1 larvae in Diptera) and by the impossibility of counting comparable aberrations when the organisms and the time of examination differ so widely. Prophase stages have frequently been found to be most sensitive (STONE 1933; MARSHAK 1935, 1937, 1939; GUSTAFSSON 1937; EKER 1937; SAX 1939). More recently metaphase and anaphase have been shown to be extremely sensitive (NEWCOMBE 1942; A. R. WHITING 1942; SPARROW 1943, 1944; BOZEMAN 1943). Drosophila work cannot be included because the mitotic stage at time of irradiation has not been determined and chromosome aberrations have not been used as criteria of sensitivity. When mutations, lethality, *etc.*, were considered, mature eggs and sperms (anaphase and resting stage, respectively) have usually been found to be most sensitive to irradiation (HARRIS 1929; HANSON and HEYS 1929; HANSON and WINKLEMAN 1929; MULLER 1930, 1940; PATTERSON, BREWSTER and WINCHESTER 1932; MOORE 1934; KOSSIKOV 1937; SONNENBLICK 1940; DEMEREC and KAUFMANN 1941).

In *Sciara ocellaris* the oöcytes not only develop synchronously, but throughout their later development (from early larval stage) possess relatively condensed, stainable chromosomes whose physical condition can be determined with considerable accuracy. The earlier studies on this species, cited above, have shown that during the long growth period the chromosomes appear to be insensitive to irradiation, but that following this period they rapidly acquire a high degree of sensitivity. In the present investigation it has been possible to correlate more exactly the cytological behavior of the chromosomes with the degree of sensitivity and apparently also with the nature of the induced rear-

rangements. The stage of meiosis of the oöcytes at the time of irradiation was inferred from the stage of eggs of control flies. Previous cytological studies have shown that within any one strain of Sciara those flies emerging on one day from sister cultures, if kept at a constant temperature after eclosion, will have oöcytes which reach a given meiotic stage at the same number of hours after eclosion of the adults. The variation in flies from sister cultures is very slight and control flies were taken from each hourly collection of flies. Sensitivity was measured by analysis of aberrations in the salivary gland chromosomes in F_1 larvae. The detectable aberrations are therefore limited to those which permit full development of the larvae.

METHODS AND TERMINOLOGY

Salivary gland slides: All examinations of F_1 salivary glands were made from permanent slides prepared by a modification of the technique of HUGHES (1939) which was adapted for Sciara by MISS JEAN LANE. Well grown larvae in which the "eye-spots" were visible were removed from the culture vials and placed in Ringer's solution. The larvae were dissected in 45 percent acetic acid and the salivary glands smeared by dropping a cover slip on the glands in a very small drop of 45 percent acetic acid. The slides were allowed to stand in 95 percent alcohol vapor for two hours. The covers were then soaked off in 95 percent alcohol and the tissue adherent to slides or covers was washed in 45 percent acetic acid, stained for five to ten minutes in aceto-carmine, washed in 95 percent alcohol, differentiated in 45 percent acetic acid and again washed in 95 percent alcohol and mounted in Diaphane.

Oöcyte slides: For oöcytes from adults more than 24 hours old, the Feulgen nucleal-reaction can be used. The method described by SCHMUCK and METZ (1931) was used. It has not been found possible to obtain a Feulgen reaction in the oöcyte nuclei of eggs from adults less than 24 hours old. For the determination of the condition of the chromosomes in eggs of these younger adults smears were made in the following manner. The flies were dissected in Ringer's solution and a few eggs were removed from one ovary and placed in a small drop of Ringer's solution on a slide. With a very fine needle each egg was broken and smeared out from the drop of Ringer's. Five percent formaldehyde was dropped on the slide immediately. Following a 10–20 minute fixation in the formaldehyde the slides were treated with Gilson's fixation fluid for 20 minutes. They were then thoroughly washed in tap water and stained with Heidenhain's haematoxylin or Ehrlich's haematoxylin.

Collection of adults: For each experiment closely related cultures were selected and were kept under constant observation during the collection of adults so that there might be no question of the virginity of the females. Sciara adults usually emerge before noon, so most of the collections were made in the morning. Immediately after eclosion adult females were shaken without etherization into fresh vials. Virgins were collected for one hour periods and stored at a constant temperature of 23°C. The mid-point of the hour was taken as the age for the entire group so that the age of each fly was known $\pm\frac{1}{2}$ hour.

Treatment: For irradiation the females were etherized and one female was removed from each group for determination of the meiotic stages of her oöcytes. The others were placed in a small dish filled almost to the brim with agar. They were then covered with gauze held down with a brass ring. All the flies were at the same level and so received approximately the same dosage.

Treatment was administered at the Radiation Therapy Department of the Hospital of the UNIVERSITY OF PENNSYLVANIA through the kindness of DR. PHILIP J. HODES and his staff. Treatments of about 1100 r were given at 25 cm target distance with a tube operated at 200 kV and 15 mA, delivering about 265 r/min; $\frac{1}{2}$ mm copper and 1 mm aluminum filters were used. Twenty-four experiments involved use of wild-type stock. In twenty-two of these the flies were given 1088 r at the rate of 277 r/min; the other two received 1116 r at the rate of 248 r/min. Sixteen experiments involved use of yellow stock. In nine of these the treatment was 1089 r at 242 r/min; in the remaining seven it was 1116 r at 248 r/min.

The control females were dissected at the time the experimental flies were being irradiated. After irradiation the treated females were again etherized and placed in individual culture vials with several vigorous young males.

Stocks: Two strains of *S. ocellaris* were used in these experiments; one wild type (Hammonds) and one yellow, a sex linked mutant body color. The data concerning the two types are analyzed separately as there is a slight difference in timing and cytological appearance of the meiotic divisions.

Terminology etc.: It is necessary in the present paper to designate individually the period of relatively rapid shortening and movement of the oöcyte chromosomes during which they pass from their position near the periphery of the nucleus to the metaphase plate of the first division spindle. In the absence of any generally accepted term for this period we refer to it here as "prophase" and refer to the earlier stages as "pre-prophase." The latter term thus becomes comparable with "early meiotic prophase" as used by many authors who include under "prophase" all the stages from leptotene to metaphase of the first division (see, *e.g.*, SPARROW whose work is discussed below).

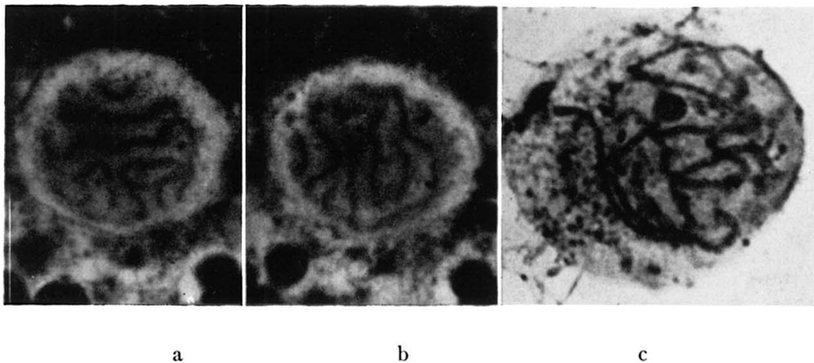
Since we consider it improbable that X-rays cause actual chromatid breaks directly we use the term break in a qualified sense and put it in quotation marks.

Owing to the fact that the data presented here do not involve large numbers, statistically, percentages are given for the most part in round numbers, omitting decimals. This introduces slight discrepancies in some of the totals, but avoids the implication of greater statistical significance than is warranted. The actual numbers upon which the calculations are based are given in the tables.

OBSERVATIONS

Oögenesis in Sciara ocellaris

The development of the oöcytes in *S. ocellaris* has been described by BERRY (1941) and a more exact determination of the condition of the chromosomes in the later stages was made by METZ and BOZEMAN (1940). The germ cells de-



a b c

FIGURE 1.—(*a, b, c*—left to right) Photomicrographs of oöcyte chromosomes of *Sciara ocellaris* Comst. during the late growth stage ("insensitive" period) shortly before the beginning of the first meiotic division. From nuclei dissected out of the eggs and fixed and stained directly on the slide, without embedding or sectioning (see METZ and BOZEMAN 1940). Fixed in formalin, followed by Gilson's fluid; stained in Ehrlich's haematoxylin.

a and *b*, upper and lower focal levels, respectively, in one intact nucleus showing the relative positions and spacing of the chromosomes (tetrads). *c*, a similar nucleus, crushed and flattened, showing better the detailed appearance of the chromosomes. Original magnification ×1700; reproduced same size. See CROUSE 1943, plate 8, figs. *n* and *o* for examples in another species.

FIGURE 2.—Metaphase chromosome group from germ line of *Sciara ocellaris*. Drawing by DR. H. V. CROUSE.

velop synchronously during the entire life of the individual, that is, from the fifth cleavage, when they reach the posterior pole plasm, until deposition of the eggs which are then in anaphase of the first meiotic division. Following segregation of the primordial germ cells a few divisions occur at the pole, after which the oögonia do not divide again until about the second larval instar. DUBOIS (1932) has described the migration of the germ cells from the posterior end of the egg to the future site of the gonads. There the germ cells are surrounded by somatic follicle cells. During the third, fourth and fifth days of larval life the oögonial divisions occur and on about the sixth day one-half the oögonia differentiate into nurse cells and one-half into primary oöcytes. Synapsis occurs early in the development of the primary oöcyte nuclei and the paired chromosomes then become arranged at the periphery of the nucleus in the form of long easily stained threads. Photomicrographs of this stage are shown in figure 1. This condition persists for two weeks, with some increase and later a decrease in nuclear volume but without any apparent movement of the chromosomes. The nurse cell nuclei increase tremendously in volume and at pupation one nurse cell and one oöcyte nucleus become surrounded by a number of follicle cells to form each egg. Ordinarily about 50–100 eggs are formed in each ovary. Growth of the nurse cell nucleus continues and a large amount of yolk is formed.

The nurse cell nucleus occupies the anterior end of the egg; and the oöcyte nucleus lies near the periphery of the egg, close to the nurse cell nucleus in young eggs, and about one-third of the way down from the anterior end in fully developed eggs. After eclosion of the adult the nurse cell gradually disappears.

When preparations are stained with haematoxylin no significant change is apparent in the condition of the chromosomes from their first appearance as condensed threads until the maturation divisions begin. That some change in viscosity of the nucleoplasm or in relative density of chromosomes and nucleoplasm, occurs at the time of pupation is indicated by the fact that the chromosomes may be displaced by mild centrifugation before, but not after, pupation. Some chemical change also occurs, for the chromosomes stain deeply with the Feulgen method at all times after differentiation of nurse and oöcyte nuclei except during a twenty-four hour period immediately following eclosion of the adult. Therefore, although no movement of oöcyte chromosomes seems to occur during the growth of the egg, the condition of the chromosomes is not static.

The long period of apparently arrested prophase is followed at about 26 hours after eclosion by a relatively rapid condensation and movement of the chromosomes to the spindle. No clear metaphase plate is formed and apparently anaphase separation begins as soon as the chromosomes are fully condensed. Anaphase movement is arrested when the dyads are separated by a distance of about one-half the length of the rods. This occurs at about 29 hours after eclosion and no further change is then seen in the chromosomes until the eggs are laid at 60–72 hours after the females have emerged. Fertilization occurs as the eggs are laid. The females die within a few hours after deposition of the eggs.

Effects of Irradiation

Percentage of oöcytes affected

The accuracy of the determination of the stage of the oöcytes at the time of irradiation depends upon the degree of synchrony of development of the oöcytes of the treated females and those of the controls and also upon the synchrony within any one female. In the control slides examined, the eggs in prophase, mid-anaphase and late anaphase showed complete synchrony within any one slide, but when grouped by cytological stages there is some overlapping of chronological age groups, especially in experiments conducted on different days. The cytological stage of the control is used even when there is some discrepancy in actual ages. Classification at metaphase and early anaphase is more difficult because these stages are of very short duration and hence do not exhibit as complete synchrony within an individual slide.

The numbers of F_1 slides examined are in many cases small because of the extremely low fertility of flies treated at these sensitive stages. As already noted, two strains of flies were used in the experiments, one a wild type strain, the other a body color mutant strain, "yellow."

The data summarized to indicate the percentage and number of slides showing rearrangements are given in table 2 and figure 3. In the wild-type flies

treated when the oöcytes were in prophase the number of F_1 slides containing aberrations is ten percent of the total. Of the five affected slides three were from one culture vial and may have unduly affected the percentage found in prophase. In the material treated at metaphase 30 percent of the slides showed aberrations. Slides from early anaphase treatments show a similar sensitivity with 30 percent of the slides having aberrations. The mid and late anaphase groups are very nearly alike with 49 percent and 50 percent of aberrations respectively.

Larger numbers of animals were examined among the offspring of the treated yellow flies (table 2). The percentage of slides showing aberrations in each of

TABLE 2

Percentage of salivary gland slides with rearrangements. Summary of the data including both wild-type stock and yellow stock. Figures in parentheses indicate numbers of slides examined. Dosage 1088–1116 r.

STAGE AT TIME OF TREATMENT	PERCENTAGE OF SLIDES WITH REARRANGEMENTS		
	WILD	YELLOW	BOTH STOCKS
Prophase	10 (50)	3 (211)	4 (261)
Metaphase	30 (43)	22 (9)	28 (52)
Early Anaphase	30 (23)	— —	30 (23)
Mid Anaphase	49 (35)	52 (60)	51 (95)
Late Anaphase	50 (20)	38 (138)	40 (158)

the stages is similar to the percentage found in the wild-type series. In prophase 3 percent of the slides examined showed aberrations; in metaphase 22 percent; in mid-anaphase, 52 percent; and in late anaphase, 38 percent.

In all cases except mid-anaphase the wild-type race shows a slightly higher frequency of affected individuals than does the yellow race. Whether this difference is due to an actual difference in sensitivity, to age grouping which is not exactly comparable, or to errors of sampling, has not been determined. The results of these experiments (table 2) are shown graphically in figure 3.

Sensitivity of oöcytes is extremely low in the prophase stage in the young adults treated here, and is even lower (METZ and BOCHE 1939, METZ and BOZEMAN 1942) in younger adults, pupae and larvae. The number of affected individuals increases in eggs treated in metaphase. For the wild type race, for which some data are available, early anaphase is similar to metaphase and sensitivity increases still more in mid and late anaphase. Further work would

be necessary to ascertain the exact decrease in sensitivity which seems to occur at late anaphase in the yellow strain.

In the wild race, late anaphase, where 50 percent of the slides are affected, is five times as sensitive as prophase where only 10 percent are affected. In the yellow race mid-anaphase, where 52 percent of the slides were affected, is 18 times as sensitive as prophase. For the two strains mid-anaphase is 12 times as sensitive as prophase. This, however, is only an approximation to the total

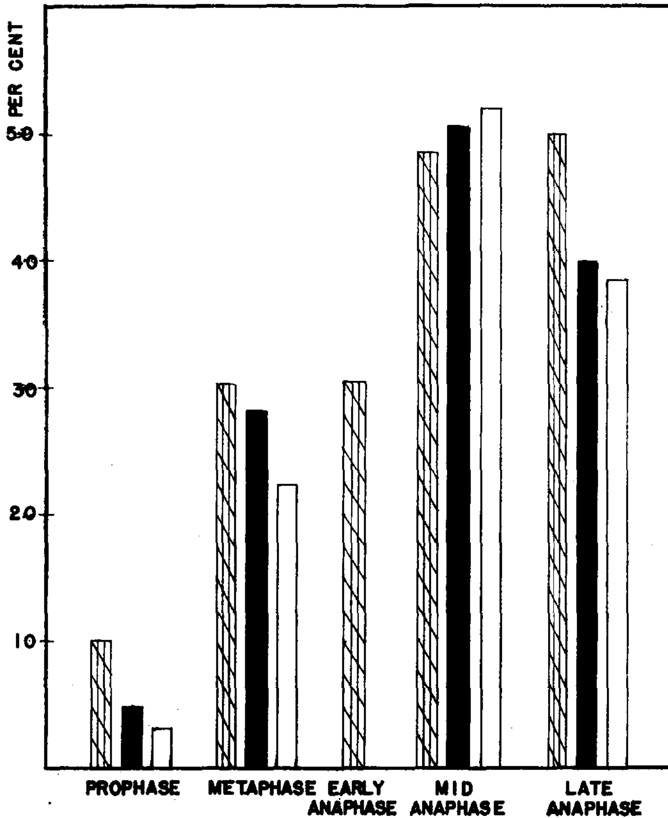


FIGURE 3.—Percentage of salivary gland slides with rearrangements. Data given in table 2. Left column (diagonally striped) represents calculations based on wild stock; open column at right calculations based on yellow stock; solid black column based on totals of both stocks.

increase in sensitivity as earlier prophase stages are almost completely insensitive (METZ and BOCHE 1939; METZ and BOZEMAN 1942).

Number of affected loci

The analysis of the number of affected loci per treated cell is somewhat difficult as there may be more than one way to interpret the number of loci involved in certain aberrations, especially in those aberrations containing repeated regions.[2] The nature of induced repeats will be discussed later. In most

[2] Induced repeats have been found with surprising frequency in our experiments.

cases they may be counted as being due to either two or three hits or "breaks."[3] For the present, both methods of interpretation are used and separate calculations are made—one on the assumption that for each repeat two loci are affected, the other on the assumption that three loci are affected in each case. The method of calculation makes little difference in the general picture when the number of affected loci per treated cell is determined, but makes a striking difference when the number of affected loci per affected cell is considered. The data for the number of affected loci per treated cell are summarized in table 3

TABLE 3

Number of affected loci per treated cell. Lower numbers, in parentheses, include repeats calculated as three "breaks"; upper figures as two "breaks."

STAGE	WILD	YELLOW	BOTH
Prophase	.20 (.28)	.06 (.07)	.08 (.11)
Metaphase	.67 (.81)	.44 (.44)	.60 (.69)
Early Anaphase	.73 (.78)	— —	.73 (.78)
Mid Anaphase	1.51 (1.57)	1.63 (1.80)	1.59 (1.72)
Late Anaphase	1.45 (1.70)	1.01 (1.24)	1.07 (1.30)

and are represented graphically in figures 4 and 5. The increase in sensitivity from prophase to mid-anaphase appears to be even more pronounced when the number of affected loci is considered than when the number of affected larvae is used as the criterion of sensitivity (table 2).

The discrepancy between the results from the wild and yellow strains is more pronounced when the "breaks" per cell are counted than when affected larvae are counted. These differences might be exaggerated by the small samples in certain groups.

When the number of affected loci per affected cell is calculated (table 4) a somewhat different picture is obtained if repeats are considered to involve two loci rather than three. Calculating repeats as two "breaks" presents a picture more nearly like that given in the previous analyses where there is a rise from prophase through mid-anaphase and then a slight drop at late anaphaes (fig. 6). Calculating repeats as being due to three "breaks" gives a very similar value for prophase, metaphase and early anaphase with an increase at mid-anaphase

[3] That is, the "breaking" of two chromatids at the same locus may be regarded as a single event, due to one hit, or as separate events due to two hits. In the former case a repeat would involve two hits or "breaks," in the latter three (see below).

and late anaphase (fig. 7). This lack of consistency with the other analyses may lend some weight to the argument that repeats do not involve three loci.

Number of loci involved

The interpretation of the number of loci involved in each aberration is complicated, as were the preceding analyses, by the uncertainty regarding the number of loci involved in repeats. A classification of the affected individuals according to the total number of loci involved in their rearrangements is shown in table 5. The same data classified on the basis of percentage are represented in table 6. It is evident from these and preceding tables that there must be an

TABLE 4

Number of affected loci per affected cell. Upper figures include repeats calculated as involving two loci. Figures in parentheses include repeats calculated as involving three loci.

STAGE	WILD	YELLOW	BOTH
Prophase	*2.00*	*2.00*	*2.00*
	(2.80)	(2.50)	(2.63)
Metaphase	*2.23*	*2.00*	*2.20*
	(2.69)	(2.00)	(2.60)
Early Anaphase	*2.42*	—	*2.42*
	(2.57)	—	(2.57)
Mid Anaphase	*3.11*	*3.16*	*3.14*
	(3.23)	(3.48)	(3.39)
Late Anaphase	*2.90*	*2.64*	*2.68*
	(3.40)	(3.22)	(3.25)

increase from prophase to anaphase in the total number of loci involved. Not only are there more cells with more than two "breaks" as mitosis progresses but the highest number of "breaks" is greater in the later stages.

Types of aberrations

A classification of the data according to types of aberrations is presented in table 7. Among the possible types of aberrations detectable in salivary gland chromosome preparations no terminal deletions, "single break aberrations," were observed. These may either not occur, due to the mechanism of rearrangement, or they may be lethal to developing eggs and larvae. Only one translocation (intercalary) was observed. The low frequency of translocation is possibly due to the position of the chromosomes at the time of rearrangement. Reciprocal translocations should not be lethal unless some as yet undiscovered process is operative to cause their elimination in Sciara. Known translocations, induced by irradiating sperms (CROUSE 1943), are readily transmitted through the female as well as the male, showing that the meiotic process does not auto-

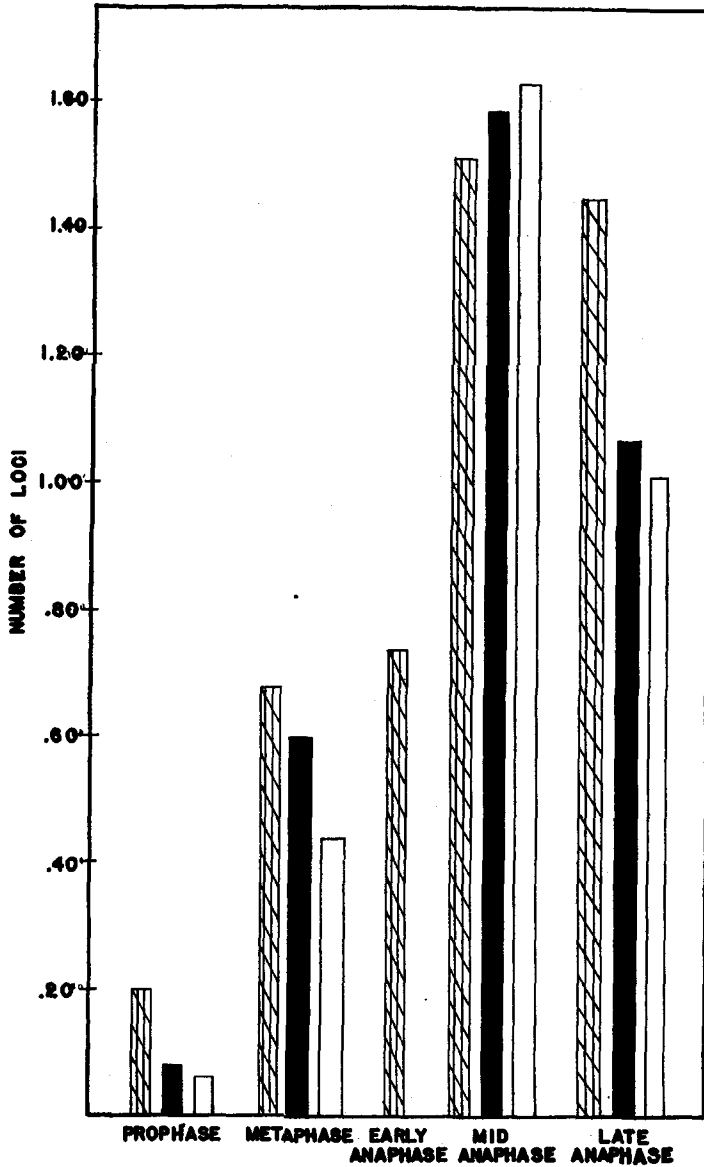


FIGURE 4.—Number of affected loci per treated cell. Repeats counted as 2 "break" rearrangements. Data given in table 3. Left column (diagonally striped) represents calculations based on wild stock; open column at right calculations based on yellow stock; solid black column based on totals of both stocks.

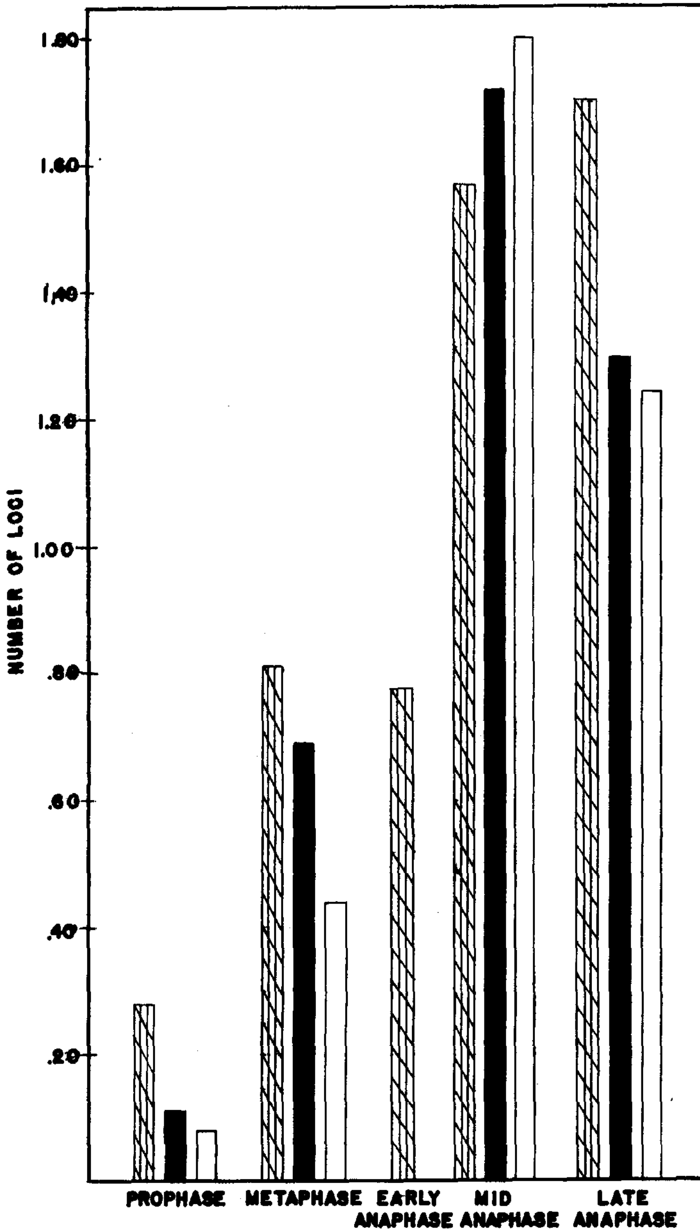


FIGURE 5.—Number of affected loci per treated cell. Repeats counted as 3 "break" rearrangements. Data given in table 3. Left column (diagonally striped) represents calculations based on wild stock; open column at right calculations based on yellow stock; solid black column based on totals of both stocks.

matically weed out such aberrations and is not necessarily upset by them. Intercalary deletions were rare, accounting for 9 percent of prophase aberrations, 25 percent of metaphase aberrations, 0 percent of early anaphase aberrations, 6 percent of mid-anaphase aberrations and 10 percent of late anaphase aberrations. Inversions show an increasing relative frequency with age at time of irradiation, forming 18 percent of all aberrations at prophase, 31 percent at metaphase, 43 percent at early anaphase, 60 percent at mid-anaphase, and 61 percent at late anaphase.

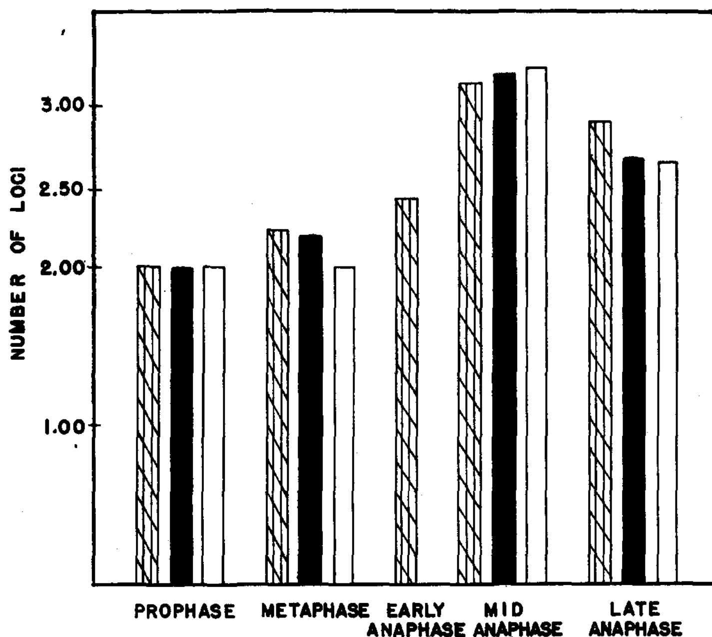


FIGURE 6.—Number of affected loci per affected cell. Repeats counted as 2 "break" rearrangements. Data given in table 4. Left column (diagonally striped) represents calculations based on wild stock; open column at right calculations based on yellow stock; solid black column based on totals of both stocks.

These experiments have produced in considerable numbers a type of aberration involving duplication of segments which has hitherto been observed rarely. KAUFMANN (1941) found the same sort of aberration in Drosophila salivary glands. The duplicated segments may occur as tandem (serial) repeats or reversed repeats, or the added segment may be inserted in some other portion of the chromosome. Of the total of 38 simple repeats detected 26 are recorded as coming in the first category (tandem), 7 in the second and 5 in the third. In the earliest stages repeats are relatively more frequent in relation to the total number of aberrations, forming 73 percent of prophase aberrations, 38 percent of metaphase aberrations, 28 percent of early anaphase aberrations, 26 percent of mid-anaphase aberrations and 20 percent of late anaphase aberrations, (see fig. 8). This decrease in relative frequency of duplications is due, however, to the increase of other aberrations, rather than to actual decrease

in the duplications themselves. In prophase 3 percent of all slides have duplications; 11 percent of all metaphase slides have duplications; 9 percent of late anaphase slides have duplications. No significant difference has been detected between the relative frequencies of direct (tandem) and reversed repeats in the different stages. The chromosome conditions determining the differences just reviewed need to be studied further, and we hope to deal with this subject in a separate paper in which the nature, mode of origin and possible significance of repeats will be considered in detail. At present it may suffice to indicate that all

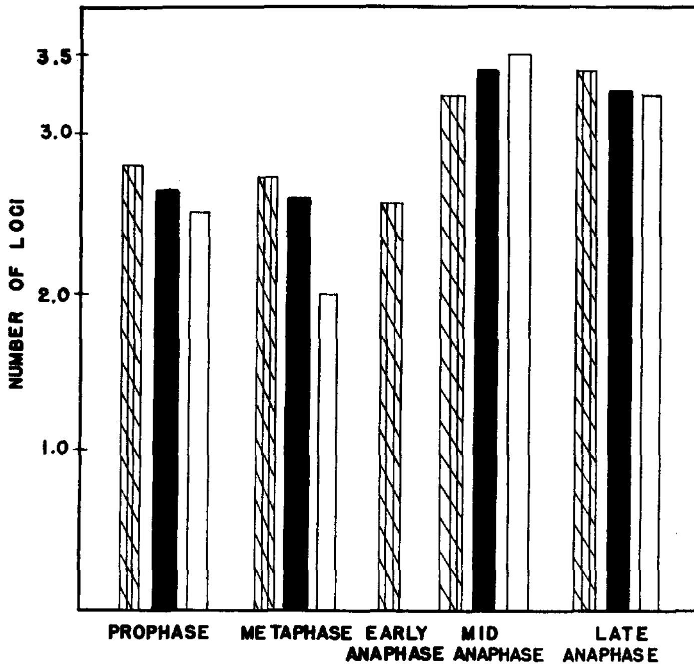


FIGURE 7.—Number of affected loci per affected cell. Repeats counted as 3 "break" rearrangements. Left column (diagonally striped) represents calculations based on wild stock; open column at right calculations based on yellow stock; solid black column based on totals of both stocks.

the repeats thus far identified in this study represent gross modifications. They are of various sizes, but each includes numerous chromatic bands. Whether or not "minute" duplications, of the type found frequently in nature in Sciara (METZ 1937) are also induced by irradiating oöcytes is not certain because they are much more difficult to detect than the gross aberrations considered here. The "minute" modifications ordinarily involved only one disk, or a pair.

Presumably the repeats and some of the intercalary deletions represent reciprocal classes derived through a common process, the donor chromosome becoming deficient for the segment contributed to the recipient chromosome. Since deficiencies may also arise in other ways, however, they are not lumped with duplications in the tables. The lower apparent frequency of deficiencies, of course, is probably due to greater lethal effects of losses than of comparable additions, in many cases.

TABLE 5

Affected individuals classified according to total number of loci involved in their rearrangements. Upper figures represent the distribution when repeats are calculated as involving two loci. Figures in parentheses represent the distribution when repeats are calculated as involving three loci.

STAGE	WILD STOCK						YELLOW STOCK						BOTH STOCKS					
	Number of loci						Number of loci						Number of loci					
	2	3	4	5	6	7	2	3	4	5	6	7	2	3	4	5	6	7
Prophase	5	—	—	—	—	—	6	—	—	—	—	—	11	—	—	—	—	—
	(1)	(4)	—	—	—	—	(3)	(3)	—	—	—	—	(4)	(7)	—	—	—	—
Metaphase	12	—	—	1	—	—	2	—	—	—	—	—	14	—	—	1	—	—
	(7)	(5)	—	—	(1)	—	(2)	—	—	—	—	—	(9)	(5)	—	—	(1)	—
Early Anaphase	5	2	—	—	—	—	—	—	—	—	—	—	5	2	—	—	—	—
	(4)	(3)	—	—	—	—	—	—	—	—	—	—	(4)	(3)	—	—	—	—
Mid Anaphase	9	3	2	1	1	1	15	3	10	1	—	2	26	6	12	2	1	3
	(7)	(5)	(2)	(1)	(1)	(1)	(9)	(9)	(6)	(5)	—	(2)	(16)	(14)	(8)	(6)	(1)	(3)
Late Anaphase	6	1	1	2	—	—	38	5	5	2	2	1	44	6	6	4	2	1
	(3)	(4)	—	(3)	—	—	(31)	(11)	(4)	(4)	(2)	(1)	(34)	(15)	(4)	(7)	(2)	(1)

TABLE 6

Same data as given in table 5 but showing percentages rather than total numbers of affected individuals in the different classes. (See legend of table 5.)

STAGE	WILD STOCK						YELLOW STOCK						BOTH STOCKS					
	Number of loci						Number of loci						Number of loci					
	2	3	4	5	6	7	2	3	4	5	6	7	2	3	4	5	6	7
Prophase	100	—	—	—	—	—	100	—	—	—	—	—	100	—	—	—	—	—
	(20)	(80)	—	—	—	—	(50)	(50)	—	—	—	—	(36)	(64)	—	—	—	—
Metaphase	92	—	—	8	—	—	100	—	—	—	—	—	93	—	—	7	—	—
	(53)	(38)	—	—	(8)	—	(100)	—	—	—	—	—	(60)	(33)	—	—	(7)	—
Early Anaphase	71	29	—	—	—	—	—	—	—	—	—	—	71	29	—	—	—	—
	(57)	(43)	—	—	—	—	—	—	—	—	—	—	(57)	(43)	—	—	—	—
Mid Anaphase	53	17	11	6	6	6	48	10	32	3	—	6	50	12	25	4	3	6
	(41)	(29)	(11)	(6)	(6)	(6)	(29)	(29)	(19)	(16)	—	(6)	(33)	(29)	(16)	(12)	(2)	(6)
Late Anaphase	60	10	10	20	—	—	72	9	9	4	4	2	69	9	9	6	3	1
	(30)	(40)	—	(30)	—	—	(58)	(20)	(8)	(8)	(4)	(2)	(54)	(24)	(6)	(11)	(3)	(1)

TABLE 7

Data classified according to types of aberrations.

	ADJACENT REPEATS	NON-ADJACENT REPEATS	COM-PLICATED REPEAT	INVERSION	DELETION	TRANS-POSITION	TRANS-LOCATION	TOTAL ABBERA-TIONS	TOTAL LARVAE EXAMINED
Prophase									
Yellow	3	—	—	2	1	—	—	6	211
Wild	4	—	1	—	—	—	—	5	50
Both	7	—	1	2	1	—	—	11	261
Percent	64%	—	9%	18%	9%	—	—	—	
Totals		73%			27%				
Metaphase									
Yellow	—	—	—	1	1	—	—	2	9
Wild	6	—	—	4	3	1	—	14	43
Both	6	—	—	5	4	1	—	16	52
Percent	38%	—	—	31%	25%	6%	—	—	
Totals		38%			62%				
Anaphase (early)									
Wild	1	—	1	3	—	1	1	7	23
Percent	14%	—	14%	43%	—	14%	14%	—	
Totals		28%			57%				
Anaphase (mid)									
Yellow	10	2	2	26	4	2	—	46	60
Wild	2	2	—	16	—	4	—	24	35
Both	12	4	2	42	4	6	—	70	95
Percent	17%	6%	3%	60%	6%	8%	—	—	
Totals		26%			74%				
Anaphase (late)									
Yellow	10	1	1	42	7	7	—	68	138
Wild	2	—	1	5	1	—	—	9	20
Both	12	1	2	47	8	7	—	77	158
Percent	16%	1%	3%	61%	10%	9%	—	—	
Totals		20%			80%				

The distribution of repeats among the different chromosomes is not a random one. Most of the repeats occur in chromosomes B and C; only a few are recorded for chromosome A, even though that is the longest chromosome; none was found in the X chromosome. Similarly, in the case of inversions the distribution is not at random. But there seems to be no correlation between the distribution of inversions and of repeats, except for the almost complete absence of both types in the X chromosome. Among the autosomes, chromosome A has a much higher frequency of inversions than of repeats, while chromosomes B and C exhibit a proportionately lower frequency.

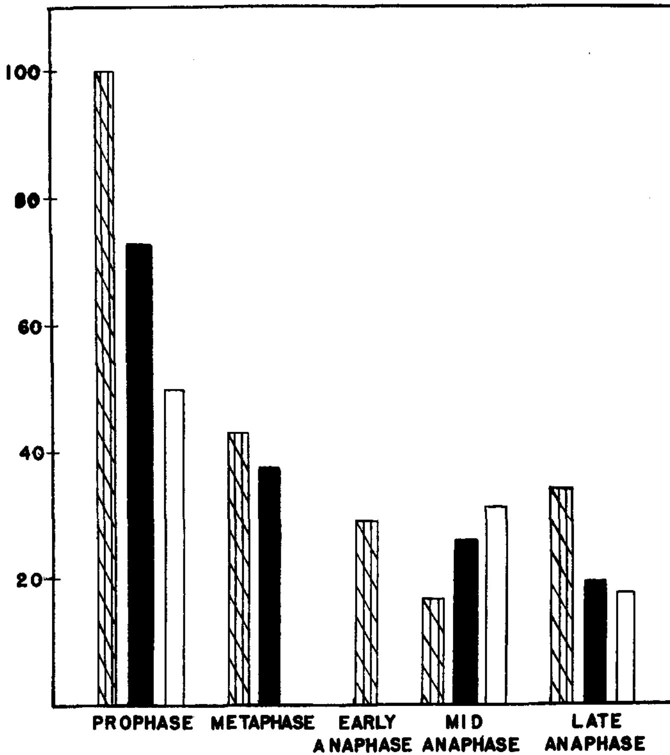


FIGURE 8.—Relative frequency of duplications expressed as percent of all aberrations. Data given in table 7. Left column (diagonally striped) represents calculations based on wild stock; open column at right calculations based on yellow stock; solid black column based on totals of both stocks.

DISCUSSION

The following discussion will deal primarily with three points: (1) the initial effects of X-radiation, (2) the time at which chromosome rearrangement occurs relative to the time of treatment, and (3) the mechanism of rearrangement. The terms "sensitive" and "insensitive" are used mainly in a purely descriptive sense; if no rearrangements or other effects are detected following treatment the chromosomes are said to be "insensitive." Whether or not they actually are insensitive will be discussed in later paragraphs.

As intimated previously, there is a period of at least two weeks preceding the onset of the first meiotic division in oöcytes of *Sciara ocellaris* during which the chromosomes appear to be insensitive to irradiation, as judged by present criteria. The main criterion is the presence or absence of rearrangements in the salivary gland chromosomes of F_1 larvae, although pycnosis or its absence in the chromosomes immediately after irradiation has also been used. During the long "insensitive" period the chromosomes are in a pre-prophase condition and lie close to the nuclear membrane. As they go from this stage into the active stages of the meiotic division they become "sensitive" and show different degrees of sensitivity as the division progresses. There is evidence also of a correlation between the nature of the modifications and the different stages treated.

We are dealing, therefore, with a long period of apparently complete insensitivity, followed by a relatively short period (so far as observed) of high sensitivity. Since the scoring is done on the fully grown F_1 larvae, ample time elapses between treatment and scoring to permit completion of all rearrangments that are induced. In other words, no induced modifications are missed because of scoring too soon after treatment.

The sharp contrast between the insensitivity of the chromosomes in the pre-prophase, and their sensitivity from prophase to anaphase, indicates that some change occurs at approximately the outset of the meiotic division which brings about sensitivity in a relatively abrupt manner, increasing in degree as the division progresses. Is this a change in the nature of the chromosomes, or in their behavior, or in other cell components?

The only conspicuous and coincident changes in other cell components are the breakdown of the nuclear membrane and the formation of the mitotic figure. The latter is presumably not a factor; but breakdown of the nuclear membrane exposes the chromosomes to cytoplasmic influence, which might well have an effect on them. Although our present evidence does not provide a suitable test, the fact that sensitivity increases with time after the nuclear membrane disappears might be interpreted as reflecting the penetration of a substance through the nucleoplasm to the chromosomes. Such an influence might act directly to bring about chromosome disturbances, as has been postulated by previous authors in other material (*e.g.* DURYEE 1939). Or it might merely serve to sensitize the chromosomes to irradiation. Our results have some significance in distinguishing between these two possibilities. On the former hypothesis it would be assumed that irradiation produces a substance in the cytoplasm which penetrates to the chromosomes and induces modifications. But our evidence indicates that irradiation before breakdown of the nuclear membrane gives no results. Therefore, either no effective substance is produced before the membrane disappears, or it does not persist long enough to influence the chromosomes after disappearance of the membrane. On the alternative hypothesis it is not necessary to assume that any substance is produced in the cytoplasm by the irradiation. The substance might be a normal component of the cytoplasm, such as an enzyme, which penetrates after the nuclear membrane disappears and modifies the chromosomes in such a way as to make them sensitive to irradiation. The work of CHAMBERS (1924),

DURYEE (1939, 1941) and D'ANGELO (1946) indicating the marked effect of cytoplasm on chromosomes after rupture of the nuclear membrane, is suggestive in this connection. Both of these hypotheses meet difficulties, of course. For example, our evidence indicates that when the prophase dissolution of the nuclear membrane occurs the chromosomes are at the periphery, close to the membrane, where they could be reached most easily and quickly by any cytoplasmic influence. Yet sensitivity increases progressively after this stage—a fact which calls for subsidiary assumptions. On the second hypothesis mentioned the assumption would be that after the sensitizing substance enters from the cytoplasm it requires time to produce its maximum sensitizing effect; hence later stages would be more sensitive than earlier ones. On the whole, this hypothesis seems to fit the data better than any other, insofar as correlation between sensitivity and mitotic stages is concerned, and we will return to it later after considering other alternatives.

Attempts to explain the sensitivity changes on the basis of chromosome behavior give no clear result. The microscopically visible evidence on this point may be summarized as follows: During pre-prophase the chromosomes are in the form of long, but well condensed, tetrads—that is, heavy, not delicate, threads. As they enter the stages of meiotic division and become sensitive they undergo at least five types of change. (1) They become much shorter and hence their constituent chromonemata presumably become more closely coiled. (2) They lose the association with the nuclear membrane, which coincidently disappears. (3) Whereas they have been lying in an apparently passive condition, evenly spaced, they now take on movement. (4) They also become subject to the tensions or stresses imposed by the spindle fibers or other polarized forces acting in the mitotic figure. (5) Although in the earlier, more elongate, condition the chromonemata have presumably been surrounded by "matrix" material, they now come to be coiled within a relatively firm bulky matrix, typical of the metaphase chromosomes.

Whether or not we may attribute the increase in sensitivity to any of these detectable chromosome changes depends in part on how and when chromosome rearrangement takes place. An interpretation based on chromosome movement, or lack of it, has been suggested in earlier papers. On this basis the failure to induce rearrangements by treatment in pre-prophase has been attributed to absence of chromosome movement during this period and consequent "healing" of the affected regions before rearrangements can be brought about (METZ and BOCHE 1939). This interpretation, as stated, would imply that rearrangements occur during or shortly after treatment (BOZEMAN 1943), rather than following a considerable period of delay. Such a view seems to be supported by the present evidence indicating that different types of rearrangements are secured with different relative frequencies following treatment at different stages. On the other hand, any assumption like this of immediate rearrangement is open to at least two serious objections. One is that the nature of the rearrangements themselves is hard to explain on this basis; *e.g.*, if rearrangement occurs immediately why are inversions and not translocations brought about by treatment during metaphase and anaphase, especially in the

case of rod-like chromosomes? The other comes from the increasing evidence of delayed rearrangement in other organisms (MULLER 1940; KAUFMANN 1941; BISPOP 1942; MCCLINTOCK 1942; SPARROW 1944).

In view of these difficulties it is desirable to examine the present evidence critically from both standpoints. Four main features appear to be of primary importance in this connection, (1) the insensitivity of the pre-prophase stages, (2) the appearance of sensitivity and its rapid increase from prophase to anaphase, (3) the absence of translocations, (4) the increasing *relative* frequency of inversions, as compared with other aberrations (deletions, duplications) from prophase to anaphase. In considering these features we are immediately confronted with the question as to what the initial effects of irradiation are on the chromosomes. If we leave out of account the possibility considered above that rearrangements are induced indirectly, through the cytoplasm, we may assume like most other workers, that the initial effects are produced directly on the chromosomes by ionizations. They would be localized modifications, therefore, which have the potentiality of leading, either immediately or later, to rearrangement or breakage of chromonemata. Because of size relations (see *e.g.* METZ 1941) we consider it very doubtful that an ionization can itself bring about actual breakage of a chromonema. We would, therefore, explain chromosome fragmentation, such as that induced in maize (*e.g.* STADLER 1931; MCCLINTOCK 1931), Tradescantia (*e.g.* SAX 1940; FABERGÉ 1940; SWANSON 1942), Trillium (*e.g.* SPARROW 1944), Orthoptera (*e.g.* WHITE 1937; CARLSON 1941; BISHOP 1942) and other organisms, as due to secondary factors of some kind, possibly mechanical, such as gross movements of the cytoplasm or nucleoplasm, which might break the chromonemata at spots weakened by the ionizations. On such a view a localized modification may either lead to a break or to a rearrangement or may be "healed," depending on circumstances. The possible nature of the localized modification will be considered in later paragraphs.

We may first examine the four lines of evidence just cited with respect to the possibility of immediate rearrangement. On this basis the insensitivity of the pre-prophase stages would be interpreted in the same general manner as before. Since the tetrads are well separated from one another, and since no evidence of movement has been detected, we would assume that if initial modifications are induced during this period they are healed before an opportunity is provided for rearrangement or breakage. Similarly the change to a sensitive condition, coincident with prophase movement, could be interpreted as due to this movement or some other change, such as the introduction of a sensitizing cytoplasmic agent, at this time. Mitotic movement alone, can hardly account satisfactorily for the increase in sensitivity that extends into the anaphase stage at which mitotic movement ceases—i.e., the stage in which the chromosomes remain apparently stationary until the egg is fertilized and laid. Apparently the chromosomes are in a condition of maximum sensitivity at this stage of arrested mitotic movement. If rearrangement occurs at approximately the time of treatment, therefore, something more than coincident movement is needed to account for it. On the other hand, as already noted, the difference in relative frequencies of inversions, as compared with other alterations, at

different stages, suggests the occurrence of immediate rearrangement, because on that basis it would not be surprising to have certain types of rearrangement favored by conditions existing at individual mitotic stages. Any alternative view would seem to require the assumption that different kinds of initial effects are produced by the irradiation and that those which lead to inversions increase in frequency more from prophase to anaphase than do those responsible for deletions and duplications. Although the mechanism responsible for duplications and deletions is somewhat different from that responsible for inversions, the difference can hardly explain the differences in frequencies just referred to if rearrangement occurs long after treatment rather than immediately. Nevertheless, as will appear below, we are inclined to the view that rearrangement may actually be delayed here.

Using *Trillium erectum* as experimental material, SPARROW (1944) has secured results similar in many respects to those reported here and earlier (BOZEMAN 1943). He investigated separately the effects of X-radiation on five different stages in microsporogenesis: leptotene-zygotene, pachytene, first metaphase, first anaphase and microspore resting stage. Cells rayed at leptotene-zygotene and pachytene were scored for aberrations at first anaphase. "Those rayed at metaphase and anaphase showed little or no increase in aberrations until microspore division which followed directly, without an intervening second meiotic division. Scoring was done at microspore metaphase following irradiation at meiotic metaphase and anaphase, and at microspore anaphase following X-ray treatment of microspores in the resting stage." (p. 148) The least sensitive stages were found to be the leptotene-zygotene and the microspore resting stage, with an increase at pachytene and a large increase at first metaphase and anaphase—the anaphase value being about eight times that of the two low stages. No stage was recorded as insensitive. The validity of the results from treatment of leptotene-zygotene and pachytene stages may be questioned, because cells so treated were examined in the immediately succeeding anaphase, rather than later in the microspore divisions. Nevertheless, the general relationship between sensitivity and stage is similar to that observed in our results. By examining treated material at intervals following treatment, SPARROW was able to show that rearrangement and fragmentation occur after considerable delay. For example, as just noted, this is true of chromosomes rayed at first meiotic metaphase and anaphase. They show no effects when examined one, two or three days later, but show a great effect when examined in microspore mitosis. Summarizing, SPARROW states that "Our results indicate that X-rays fail to break metaphase and anaphase chromosomes, as such, but that they somehow react to produce potential breaks which become visible chromatid or chromosome breaks only after the nucleus has passed through an interphase." Thus the observations support those of other recent workers cited above in indicating that rearrangement is "delayed," not "immediate."

In attempting to account for the differences in chromosome sensitivity at different stages in the cell cycle, SPARROW suggests that sensitivity is correlated with the content of desoxyribose nucleic acid in the chromosomes. This is

based on two lines of evidence: (1) "Stages, such as resting nuclei or early prophase, which have low concentrations of desoxyribose nucleotides or nucleic acid have a low sensitivity, while metaphase and anaphase, stages which have a high concentration, show a high radiosensitivity." (2) There is "evidence that small doses of X-rays can temporarily block one or more of the essential processes involved in the nucleic acid cycle." As SPARROW points out, however, the content of nucleic acid may not be the determining factor *per se* but "may be merely concomitant."

If the Feulgen nucleal reaction is a reliable criterion for determining the presence of desoxyribose nucleic acid, our results are difficult to interpret on the basis of SPARROW's suggestion. Although, as judged by this test, there is a high concentration of desoxyribose nucleic acid during the most sensitive stages (metaphase and anaphase) there is a similar high concentration during most of the long, insensitive pre-prophase. This concentration persists through the period just mentioned, which is insensitive, then disappears for about the first 24 hours after emergence of the adult, likewise an insensitive period, and then reappears as the first meiotic division begins. These facts might suggest that rate of synthesis, rather than concentration, of desoxyribose nucleic acid may be the determining factor; but if this were the case, it would be natural to expect high sensitivity in prophase and relatively low sensitivity in anaphase, which is just the reverse of what is found.

On an interpretation of the kind just mentioned, it is apparently assumed that differences in the end results are due to differences in the initial effects of irradiation on chromosomes in different stages. Without implying any criticism of this view, we may outline briefly an alternative type of interpretation based on the opposite assumption—namely, the assumption that the initial effects are similar at all stages and that differences in end results are due to what happens after treatment. To simplify presentation two further assumptions are made: (1) that rearrangement is delayed, not immediate, in the case of cells treated in the active stages of mitotic or meiotic division, (2) that such rearrangement occurs at some particular stage in the cell cycle. In the latter respect rearrangement and fragmentation may differ; the former may occur at one stage and the latter at another (or others). It will be convenient to use mainly our own results for purposes of illustration.

As pointed out by BELLING (1927, 1933) and numerous others, chromosome rearrangement may involve a process akin to crossing over. The probability that it really does is increased by the growing evidence, such as that of SPARROW, indicating that irradiation produces directly only potential, not actual, breaks in the chromosome threads. Actual breaks lead to fragmentation, which is only detected after a period of delay. In our opinion rearrangement may not involve actual breakage, except in the technical sense used in connection with ordinary crossing over. Hence it is possible that fragmentation and rearrangement represent two distinct processes, although they result from the same initial effects. If we assume that the initial ionizations produce effects which weaken the chromonema we may expect that actual breaks could subsequently

occur if the chromonema were subjected to stresses and strains. Such appears indeed to be what occurs in the orthopteran material described by BISHOP (1942) where fragmentation occurs at the first meiotic division although treatment was given to spermatogonia several cell generations earlier. Chromosome rearrangement, however, would not require stresses and strains and might occur at a different time.

On the assumption commonly made, that the genic material in the chromonema, and probably the chromonema itself, is surrounded by insulating material of some sort which not only protects it, but gives it rigidity or strength, we may picture the ionizations as serving directly or indirectly to destroy or weaken this material locally, thus producing the effect leading to subsequent rearrangement or fragmentation (cf., *e.g.*, METZ 1934).

It seems reasonable to suppose that this insulating material is produced, or renewed, primarily at some one period in the cell cycle. If so, we might expect that injuries produced earlier would tend to be healed during this stage. On such a view, we might interpret the evidence reviewed above as indicating that in our material renewal occurs shortly before and during the prophase movement in the oöcyte. Disturbances produced previously, therefore, would be healed at this time if they had not already led to aberrations. Absence of rearrangements following treatment during the long pre-prophase period would thus be explained on the basis of lack of movement together with subsequent healing of the chromosome injuries. The modifications would not persist and lead to aberrations subsequent to the meiotic division itself. Treatment during the meiotic division, however, would lead to aberrations because the damaged insulating substance would not be renewed until after almost a complete cell generation had passed. Just when the aberrations would be brought about is not indicated by our results because it was not feasible to treat the eggs in telophase or interphase. We might expect rearrangements to occur, however, during interphase when the chromonemata are most extended and delicate. Possibly this gives a clue to the problem of why translocations are so rare in our material. It is well known that during interphase chromosomes may normally occupy separate regions (sometimes recognizable as vesicles) in the nucleus. This would facilitate the process of inversion and tend to prevent translocation. Fragmentation might similarly be expected at this time because of the delicacy of the threads. Fragmentation might also occur during the active stages of mitosis if the initial effects were severe enough and other conditions were suitable; but the speculative nature of this topic makes full discussion here undesirable.

SPARROW's evidence goes farther than ours on the point in question; but it likewise is insufficient to provide a satisfactory test. Following treatment of metaphase and anaphase chromosomes, breaks were not detected until after the nucleus had passed through an interphase. Apparently they may have occurred during the interphase. Two plants were rayed during the microspore resting stage and examined for aberrations in the following anaphase. Both gave a low percentage of aberrations, which tends to argue against the hypo-

thesis just suggested. Since one plant gave 1.7 times as high a percentage as the other, however, and since both may have been treated in late resting stage, the implication of the evidence is not clear.

With suitable material it should be possible to test the interpretation under consideration. What is needed is critical evidence covering late anaphase, telophase and successive stages in interphase, from favorable material.

One argument against the suggestion that rearrangements may occur during interphase in our material is seen in the data from duplications (repeats). The process of duplication requires the presence of two chromatids or chromonemata—a donor and a recipient—and leaves the donor deficient for the donated segment. If the rearrangement occurs before the second meiotic division there is no difficulty because the latter chromosome is (in the present case) eliminated through a polar body. But it is doubtful if a true, diffuse interphase condition intervenes between the first and second oöcyte divisions and, until this point is cleared up, the possibility must be recognized that the first true interphase following irradiation here is that leading up to the first cleavage division in the fertilized egg. Duplication at the latter stage would involve two sister chromonemata and would lead to a mosaic condition—one daughter nucleus containing the duplication, the other the deficiency. The deficiency might act as a cell lethal and be self-eliminating. Or it might lead to a mosaic condition which in some cases would be observable in the salivary glands. Our only direct evidence on this point is the record of one mosaic larva in our material.

In the latter part of this discussion we have ignored the hypothesis mentioned at the outset, that a cytoplasmic agent penetrates the nucleoplasm after breakdown of the nuclear membrane and sensitizes the chromosomes to X-radiation, thus explaining the sensitivity during the active stages of mitosis. This interpretation harmonizes with the increasing evidence of sensitivity at this period in other organisms (cf. SPARROW, *l.c.;* A. R. WHITING 1940, 1945). If it is correct the problems just discussed become simplified. We may postulate the same initial effects of ionizations and the same mechanism and time of rearrangement as those just considered; but we need not assume that processes of "healing" occur following treatment at non-mitotic stages, because at such stages the chromosomes would be relatively insensitive. How widely such an interpretation could be applied is not yet clear. There are obvious difficulties, such as the well known fact that in mature sperms (Drosophila, Sciara, *etc.*,) the chromosomes are highly sensitive to irradiation although the nuclear membrane is presumably intact. Possibly the relative absence of nucleoplasm and the compact clumping of the chromosomes in sperms result in a condition of sensitivity found in "ordinary" cells only at mitotic stages.

As already noted, changes in the relative frequencies of different types of aberrations induced during the meiotic division in our material are difficult to explain on the basis of delayed rearrangement. But they are also difficult to explain on any other basis in the present state of the evidence, for so far as we can see there is nothing about the nature or behavior of the chromosomes at the stages in question to give reason to expect changes of the type observed.

An extension of the study to other stages in the cell cycle may help to throw light on this aspect of the problem.

Finally, attention may be recalled to the differential distribution of rearrangements among the different chromosomes as noted on page 303. Rearrangement frequencies appear to bear no close relation to chromosome length; the frequencies of duplications do not closely parallel those of inversions; and there is considerable contrast between rearrangement frequency in the X chromosome and the autosomes. The low rearrangement frequency for the X chromosome may be due in part to the fact that about a third of the larvae examined were males—possessing, of course, only a single X in the salivary glands. But the discrepancy seems too great to account for entirely on this basis. We hope to consider these topics in a later paper.

SUMMARY

In maturing oöcytes of *Sciara ocellaris* Comst., chromosome sensitivity to X-radiation differs greatly at different stages, as measured by chromosome rearrangements recovered in the F_1 larvae. The material is particularly favorable because the oöcytes develop synchronously and the rearrangements may be observed in the salivary gland chromosomes.

Sensitivity is almost zero for a long period preceding the breakdown of the nuclear membrane at the beginning of the first meiotic division. Then it rises rapidly to a peak, in anaphase, apparently dropping off somewhat in late anaphase, at which time mitotic activity is arrested, pending fertilization.

X-ray doses of approximately 1100 r were used. Different types of rearrangements differ greatly in actual frequency. Only one translocation was secured. Inversions, duplications (repeats), deletions, and transpositions are more frequent, but their relative frequency differs at different stages. Rearrangements are not distributed at random among the different chromosomes and frequency is not closely correlated with chromosome size.

On the basis of percentage of F_1 larvae showing rearrangements, sensitivity rises from 0 percent preceding breakdown of the nuclear membrane (first meiotic division) to approximately the following: prophase, 5 percent; metaphase, 28 percent; early anaphase, 30 percent; mid-anaphase, 50 percent; late anaphase, 40 percent.

The data suggest the intervention of a cytoplasmic agent which reaches the chromosomes after breakdown of the nuclear membrane and which either induces rearrangements directly, by transmitting an influence already produced by the irradiation, or sensitizes the chromosomes to irradiation so that treatment is effective after, but not before, entrance of the agent. If the former alternative is correct, the induced cytoplasmic modification is apparently short lived, because no rearrangements are recovered after treatment during late growth stages prior to about the time of breakdown of the nuclear membrane.

A third possibility considered is that no cytoplasmic agent is involved and that the apparent differences in sensitivity are due to the intervention of a "healing" process which would "heal" the potential breaks or localized modifications before rearrangements occurred if treatment preceded the healing period. The "healing" might consist in the formation of matrix material.

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