THE PRODUCTION OF CHROMOSOME INTERCHANGES IN DROSOPHILA VIRILIS*[1]

WILLIAM K. BAKER

Department of Zoology and Entomology, University of Tennessee, Knoxville, Tennessee

Received July 6, 1948

MULLER'S original discovery that ionizing radiations are capable of breaking the chromosome thread was based on investigations carried out with *Drosophila melanogaster*. But in spite of the numerous studies which have been undertaken using this animal, comparatively little progress has been made in the last twenty years in determining the precise manner in which these breaks are formed and the mechanisms involved in the subsequent rejoining of the broken chromosome ends. The cause of this rather slow advance resides in the fact that Drosophila chromosomes can be observed satisfactorily neither during the breakage or joining process nor for a considerable interval of time thereafter. Consequently, any information regarding these processes must be obtained by inference from events taking place at a later time when the ultimate results of the radiation can be accurately determined by genetic analysis or by a cytological study of the salivary gland chromosomes.

The results of the present investigation are derived from experiments in which the sperm of *Drosophila virilis* males are subjected to different dosages of X-radiation. Only a particular type of chromosome rearrangement is studied; namely, exchanges between different chromosomes. The frequency with which these interchanges occur at the different dosage levels provides some insight into the manner in which the breaks are formed. The information obtained has been collected in such a way that both the relative sensitivity of the different chromosomes to breakage and the relative frequency with which certain numbers of chromosomes are involved in the recovered interchanges are revealed. It is possible to gain some insight into the course of events taking place during the joining of the broken chromosome ends by use of these data. Two different groups of experiments of this type have been conducted: in the one group the temperature at the time of irradiation was held at $28° \pm 1°C$, while in the other a temperature of $3° \pm 1°C$ was maintained. The finding of a significant difference in the results of these two groups merely emphasizes the inadequate state of our knowledge concerning the biological action of ionizing radiations on Drosophila chromosomes.

* Part of the cost of the accompanying tables is paid by the GALTON AND MENDEL MEMORIAL FUND.

[1] Prepared from a dissertation presented to the Faculty of the Graduate School of THE UNIVERSITY OF TEXAS in partial fulfillment of the requirements for the degree of Doctor of Philosophy. This investigation was carried out in the Genetics Laboratory of the Department of Zoology at THE UNIVERSITY OF TEXAS.

MATERIAL AND METHODS

Two methods, in general, have been used to determine the rate of induced interchange in Drosophila: the cytological method in which the salivary gland chromosomes of the F_1 larval progeny of irradiated males are examined, and the genetic method which involves an analysis of the chromosome segregation in the F_2 generation. The primary advantages of the cytological method are that, although the original number of breaks cannot be determined, the actual number of these originals breaks which were used in forming the recovered rearrangements can be detected and the exact nature of the rearrangement can be observed. The limitations of this method are centered around the fact that Y chromosome translocations and exchanges between two heterochromatic regions are difficult or impossible to detect. These limitations are quite critical since it has been shown in an extensive study by KAUFMANN (1946a) that, per unit length of salivary gland chromosome, the breaks which form rearrangements are more likely to be located in the proximal heterochromatic region than in the euchromatic regions. By use of the genetic method (used in this study), these limitations can be surmounted at the expense of detailed information regarding the recovered interchanges. The two methods should be considered complementary.

Drosophila virilis was chosen for the present investigation because this species has the simple primitive chromosome configuration of the genus. The metaphase figure as seen in the ganglion cells of the larva consists of five pairs of rods of approximately equal length and a pair of microchromosomes (chromosome 6). This makes possible the study of the interchanges formed between five strictly independent elements of nearly equal length. The wild type stock used in the experiments being reported was derived from the Pasadena *virilis* strain, while the multiple mutant stock containing broken (*b*) on the second chromosome, telescope (*t*) on the third, cardinal (*cd*) on the fourth, and peach (*pe*) on the fifth was synthesized from available mutant strains. A single pair was chosen from each of the parent strains and the offspring of this pair was the stock used throughout the study. The salivary gland chromosomes and the metaphase figures, found in the ganglion cells of the F_1 larvae, from each pair were examined and no chromosomal abnormalities were discernible. A test cross made between these stocks indicated that the chromosomes were segregating at random.

The procedure which follows was used in making the tests. In order to insure that an abundant supply of mature sperm was being treated, the X-radiation was administered to wild type males which were at least eight days old. Immediately after irradiation these males were mated in mass with mature *b*, *t*, *cd*, *pe* females. Three and one-half days after mating, the males were removed and the females were transferred to fresh half-pint culture bottles. Removal of the males was necessary to prevent the use of sperm which had not completed the maturation divisions at the time of irradiation. This time limit is a very conservative one on the basis of the results obtained by DEMEREC and KAUFMANN (1941) with *melanogaster*. Pair matings were made between

the first F_1 males to hatch from the mass cultures and females of the multiple mutant stock. The offspring of the test cross were examined to determine if the segregation of chromosomes Y, 2, 3, 4, and 5 indicates any linkage between these chromosomes. If additional information was needed to confirm the presence of an interchange, the F_2 wild type males were crossed to the mutant stock and a further count of the offspring was obtained. Cytological checks were made in certain cases in which the appearance of aneuploid classes confused the actual chromosome segregation.

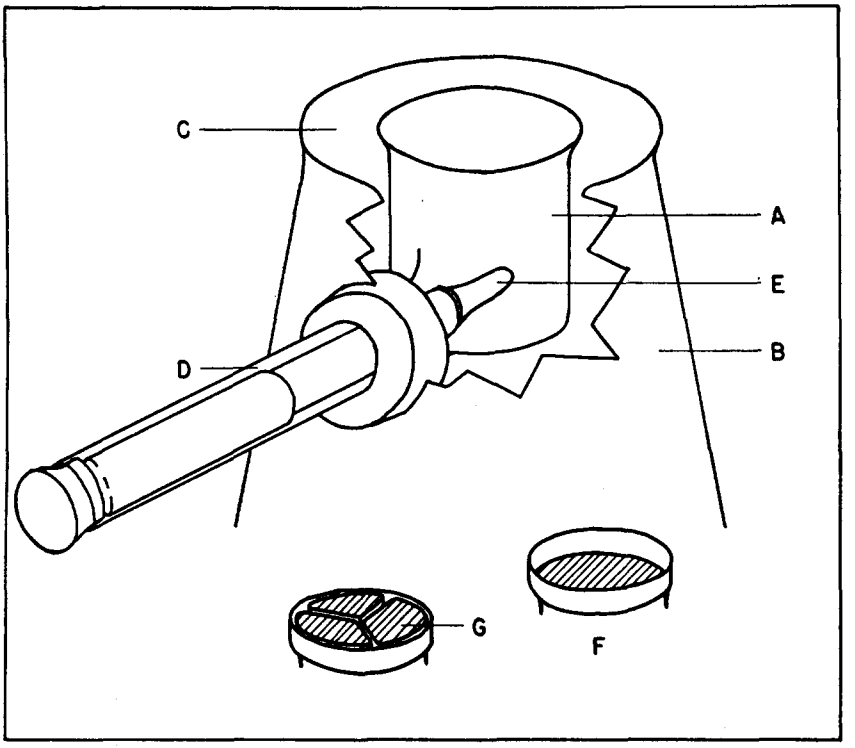

FIGURE 1. A diagram of the irradiation apparatus. A—glass irradiation chamber, B—fluid container, C—temperature controlling fluid, D—glass tube, E—ionization chamber of dosimeter, F—fly container, G—fly capsule.

In the experiments being reported, the flies were maintained at a temperature of either $28° \pm 1°C$ or $3° \pm 1°C$ during irradiation. The apparatus shown in figure 1 was used to insure comparable conditions of incident radiation and dosage measurement in the two sets of experiments. The glass irradiation chamber (A) is inserted into a metal container (B) which contains the temperature controlling fluid (C). The glass tube (D) leading into the irradiation chamber allows the ionization chamber (E) of the dosimeter to be inserted into the position where the flies are treated and also makes possible the insertion of a thermometer into the chamber while the radiation is being administered. Periodic temperature readings were made during treatment of the

flies. In the cold temperature experiments the flies were anaesthetized and placed in a container (F) which was then set on the floor of the irradiation chamber. Although the flies remained anaesthetized during treatment at the cold temperature, it was found necessary to enclose them in a capsule (G) composed of an upper and lower covering of a single thickness of gauze and sides made of light-weight cardboard during irradiation at the warm temperature. Tests show that this layer of gauze does not reduce the radiation to an extent which can be measured with the dosimeter. A fixed 1 mm aluminum filter located directly beneath the Coolidge universal X-ray tube made contact with the top of the irradiation chamber. The flies were kept, therefore, at a constant target distance during the experiment.

The X-radiation was administered to the flies in the following manner. The temperature controlling fluid—a mixture of ice and water or a continuous flow of tap water—was run into the container and maintained at a constant level. After the temperature in the irradiation chamber had become stable, the X-ray machine was calibrated by taking ten readings with a Victoreen condenser type dosimeter whose ionization chamber had been placed in the irradiation chamber. The average of these readings was used to determine the number of roentgen units the machine was delivering per unit time at the point where the flies were to receive the radiation. The flies were then anaesthetized, placed in the chamber, and 1000 r units (based on the initial calibration) were given. After this period some of the flies were removed and mated. Irradiation was continued and a sample of flies was removed and mated after each 1000 r units of treatment until flies receiving 1000, 2000, 3000, and 4000 (cold temperature experiments only) r units had been obtained. Immediately after treatment of the flies, the dosage being delivered by the machine was again checked by ten more readings with the dosimeter. The average of these two sets of readings is used as the calculated dosage administered the flies. The reading given by a condenser type dosimeter varies with temperature introducing, therefore, an instrumental error. As the temperature lowers from 22°C, the meter reads higher than the actual dosage being measured and *vice versa*. Thus in the cold temperature experiments the dosage given is somewhat less than that measured (maximum error is six percent), while in the warm temperature runs the actual dosage is slightly higher.

RESULTS

Before considering the results themselves, it should be recalled that the genetic method of analysis places certain limitations on the type of information which can be obtained. The only rearrangements that are detected are those formed in a sperm in which two or more breaks have occurred in different chromosomes. Furthermore, the resulting interchange of chromosome segments must be such that a viable, fertile zygote is formed. It is obvious that there is no possibility of directly determining the number of breaks produced in a sperm per roentgen unit of radiation. It is possible, however, to determine the minimum number of breaks which were induced in a sperm with a detect-

able interchange since the chromosomes involved in the translocation are known.

Over 800 translocations involving either the Y chromosome or the major autosomes were obtained in this study; therefore, it is not possible to give a complete tabulation of the experimental conditions under which each of these interchanges was produced. However, in table 1 will be found a summary of the number of translocations of different types (in as far as they could be classified by genetic analysis) recovered in all the experiments. The lumping to-

TABLE 1

Number of interchanges recovered of different types.

TYPE OF INTER-CHANGE	DOSAGE IN r UNITS					TYPE OF INTER-CHANGE	DOSAGE IN r UNITS				
	1000	2000	3000	4000	TOTAL		1000	2000	3000	4000	TOTAL
Y-2	3	8	15	7	33	Y-2-3-4	—	—	1	—	1
Y-3	1	8	21	3	33	Y-2-3-5	—	—	1(2)	1	2(2)
Y-4	4	3	11	7	25	Y-2-4-5	—	—	1	1	2
Y-5	4	10	15	12	41	Y-3-4-5	—	1	1(1)	1	3(1)
2-3	14(2)	20	39	22	95(2)	Y-2 & 3-4	—	—	1	—	1
2-4	8	21	36(1)	16	81(1)	Y-2 & 3-5	—	—(1)	—	—	—(1)
2-5	11	28	45	27	111	Y-2 & 4-5	—	—	1	—	1
3-4	7	28	40(1)	12	87(1)	Y-3 & 2-4	—	—	1	—	1
3-5	11	28	42	18	99	Y-4 & 2-3	—	—(1)	—	—	—(1)
4-5	16	26	29	24	95	Y-4 & 3-5	—	—	1	—	1
Y-2-3	—	1(1)	4(1)	2	7(2)	Y-5 & 2-4	—	2	—	1	3
Y-2-4	—	—(1)	1(2)	—	1(3)	2-3-4-5	—	—	1(1)	1(1)	2(2)
Y-2-5	—	1	2	—	3	2-3 & 4-5	—	1	—	1	2
Y-3-4	—	—	3	—	3	2-4 & 3-5	—	—(1)	1	1	2(1)
Y-4-5	—	1	2	—	3	2-5 & 3-4	—	2	1(1)	1	4(1)
2-3-4	—	3(1)	4(1)	7(1)	14(3)	Y-2-3-4-5	—	—	—(1)	—	—(1)
2-3-5	—	1	8	8	17	Y-2 & 3-4-5	—	—	—	1	1
2-4-5	—	—(1)	5(2)	7(1)	12(4)	Y-5 & 2-3-4	—	—	—(1)	1	1(1)
3-4-5	—	5	10(3)	5(2)	20(5)	Y-4-5 & 2-3	—	—	1	—	1

Note: The figures in parentheses are those cases, not checked cytologically, in which the survival of aneuploid classes confused the chromosome segregation to a limited extent.

gether of the data from both the warm and cold temperature experiments is permissible since the relative frequencies of the different types of translocations induced in the two sets of experiments are essentially the same. The figures given in parentheses are those cases, which were not checked cytologically, in which the survival of aneuploid classes tended to confuse the chromosome segregation to a limited extent. It can be ascertained from the data in this table that chromosome 2 is involved in 398 of the translocations, chromosome 3 in 394, chromosome 4 in 367, chromosome 5 in 423, and the Y chromosome in only 167 of the recovered interchanges. It is to be noted that the inclusion of the figures given in parentheses does not alter appreciably the relative frequency with which these chromosomes are included in an interchange. Consequently, it can be stated as a first approximation that each of the major autosomes is about equally likely to be involved in a given interchange.

The principal information obtained in these experiments is tabulated in table 2. Several things should be noted about the data which are set forth in

TABLE 2

Relation of interchange production to dosage and temperature

EXP. NO.	CHAMBER TEMP. IN °C	DOSAGE IN r UNITS (ca.)	a NUMBER OF SPERM TESTED	b NUMBER OF SPERM WITH INTER-CHANGES	c MINIMUM NO. OF BREAKS IN TESTED SPERM	d b/a	e c/a	f c/b
2	1.7–2.3	1000	140	5	10	0.036	0.071	2.000
		2000	89	10	20	0.112	0.225	2.000
		3000	108	31	65	0.287	0.602	2.097
4	2.3–3.0	982	116	4	8	0.034	0.069	2.000
		1964	115	15	31	0.130	0.270	2.067
		2946	55	17	39	0.309	0.709	2.294
		3928	73	29	64	0.397	0.877	2.207
6	2.0–2.2	1004	129	4	8	0.031	0.062	2.000
		2008	86	8	16	0.093	0.186	2.000
		3012	76	13	31	0.171	0.408	2.385
		4016	95	37	84	0.389	0.884	2.270
7	2.5–2.7	987	195	11	22	0.056	0.113	2.000
		1974	194	33	75	0.170	0.387	2.273
		2961	145	48	105	0.331	0.724	2.188
8	2.0–2.3	1027	184	5	10	0.027	0.054	2.000
		2054	187	26	59	0.139	0.316	2.269
		3081	153	49	110	0.320	0.719	2.245
		4108	145	60	145	0.414	1.000	2.417
9	3.0	997	182	14	28	0.077	0.154	2.000
		1994	182	30	64	0.165	0.352	2.133
		2991	161	52	116	0.323	0.720	2.231
		3988	75	36	82	0.480	1.093	2.278
10	2.0–2.5	987	184	10	20	0.054	0.109	2.000
		1974	168	25	51	0.149	0.304	2.040
		2961	155	48	116	0.310	0.748	2.417
		3948	74	30	66	0.405	0.892	2.200
Comb. cold exps.	1.7–3.0	998	1130	53	106	0.047	0.094	2.000
		1996	1021	147	316	0.144	0.310	2.150
		2986	853	258	582	0.302	0.682	2.256
		4016	462	192	441	0.416	0.955	2.297
11	27.5–28.2	1020	182	10	20	0.055	0.110	2.000
		2040	190	22	47	0.116	0.247	2.136
		3060	150	37	84	0.247	0.560	2.270
12	27.8–28.0	996	185	8	16	0.043	0.086	2.000
		1992	159	22	52	0.138	0.327	2.364
		2988	171	33	70	0.193	0.410	2.121
13	27.9–28.3	1011	183	10	20	0.055	0.109	2.000
		2022	167	14	29	0.084	0.174	2.071
		3033	156	34	77	0.218	0.494	2.265
Comb. warm exps.	27.5–28.3	1009	550	28	56	0.051	0.102	2.000
		2019	516	58	128	0.112	0.248	2.207
		3025	477	104	231	0.218	0.484	2.221

this table. In column c, which is a summation of the minimum number of breaks involved in the observed interchanges, the figures are equal to the sum of the number of chromosomes included in the translocations. Thus if it was found that chromosomes 2, 3, and 5 were linked, it is obvious that at least three breaks must have been induced. Considering column d, it can be seen that upon multiplication of the figures in this column by 100, the percent of the tested sperm containing detectable interchanges is obtained. These data have been plotted in figure 2. The minimum number of detectable breaks per tested sperm is recorded in column e and plotted in figure 3. The average minimum number of breaks in the recovered interchanges is compiled in the

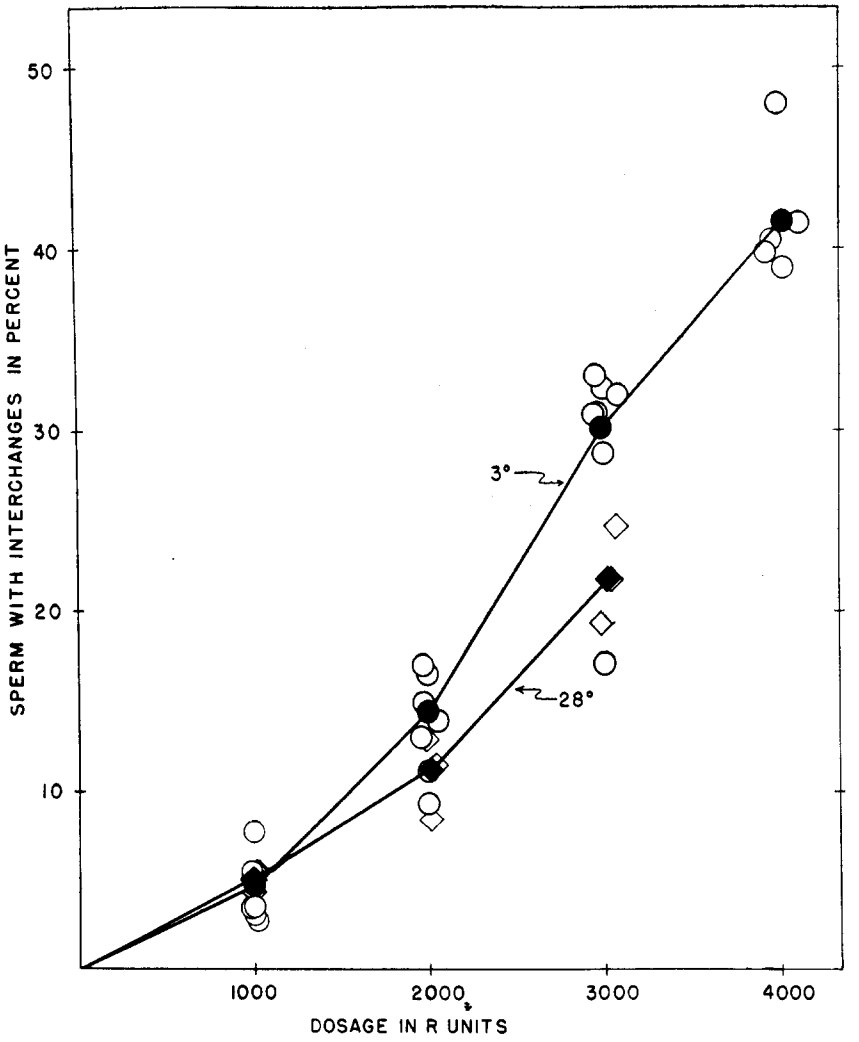

FIGURE 2. The dosage-frequency relation of the percent of sperm with translocations. Circles represent experiments conducted at 3°C, diamonds—28°C. Open symbols represent individual experiments, closed symbols—all experiments combined.

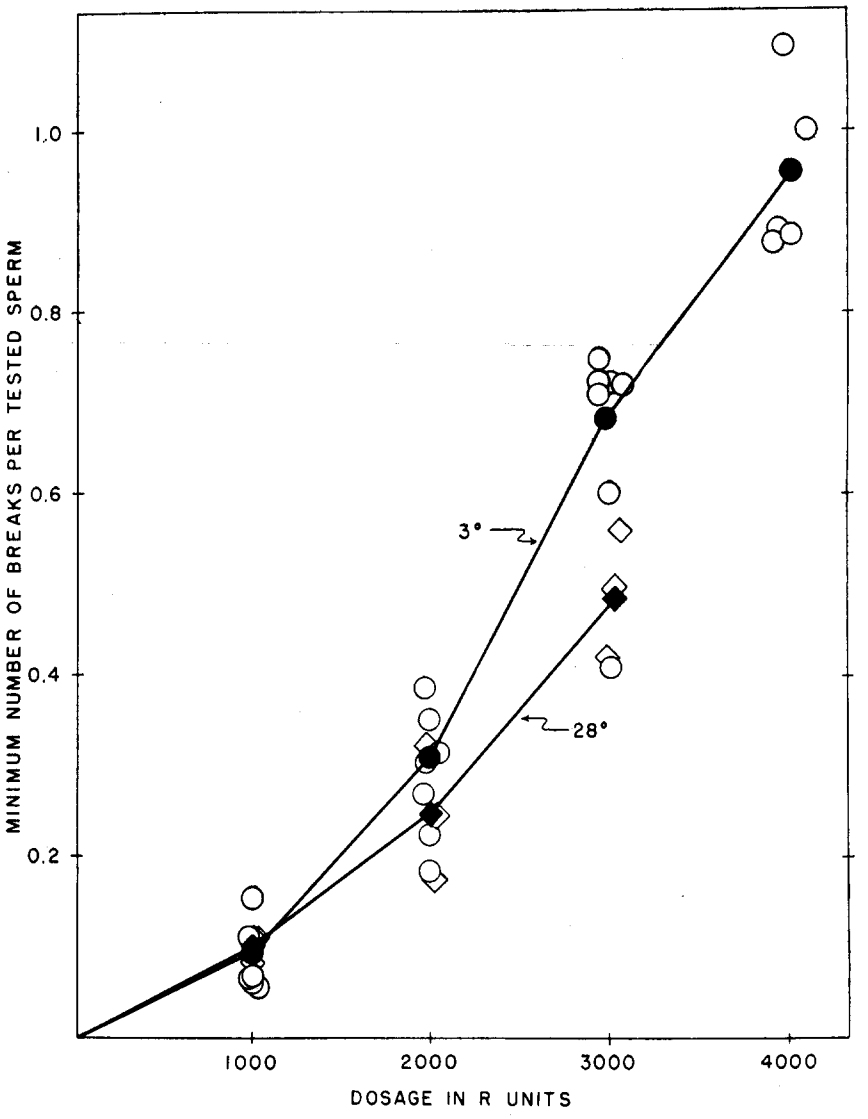

FIGURE 3. The dosage-frequency relation of the minimum number of breaks per tested sperm. See figure 2 for explanation of symbols.

last column of the table. This figure is a measure of the complexity of the rearrangement. The combined results of the individual cold and warm temperature experiments are tabulated below the data given for experiments 10 and 13, respectively. The combined data are represented by solid symbols in figures 2 and 3 while the results of the individual experiments are shown in open symbols.

The data presented in table 2 will be considered first from the standpoint of the relation between dosage and translocation production. From an examination of the sigmoid-shaped curves, for the cold temperature experiments as

presented in figures 2 and 3, it is obvious that over this range of dosage a power function of the form $y = ax^b$ does not adequately fit the empirical results. However, up to 3000 r units in both the warm and cold temperature experiments, the data approximate a power function. By use of the method of least squares, it is found that the exponent of the power curve which best fits the data plotted in figure 2 up to 3000 r units is 1.7 for the experiments performed at 3°C and 1.3 for the 28°C experiments, while for the results represented in figure 3, the exponents are 1.8 and 1.4 respectively. Care should be taken in attributing any broad significance to the particular exponent since it will be apparent from the analysis to follow that it is a function of the temperature and the number of chromosomes being followed as well as a function of dosage and the processes of breakage and union.

The data also show a significant decline in the number of interchanges produced when the temperature of the chamber in which the flies are irradiated is raised 25°C. A chi-square test of independence gives χ^2 values of 2.9 and 11.0 for the pooled data from the experiments in which the treatments were 2000 and 3000 r units respectively. (A chi-square test gives no indication of heterogeneity among the individual experiments at these dosages.) Such values or larger values of chi-square would be expected in less than one out of twelve samples at 2000 r units and less than one out of a thousand samples at 3000 r units if the temperature difference was ineffective. This difference is even more significant than the figures indicate since the dosimeter reads higher than the actual dosage when it is placed in the irradiation chamber held at 3°C. Thus the dosage delivered in the cold experiments is somewhat less than the calculated figure. This temperature effect may be summarized by stating that fewer interchanges are recovered when the sperm are treated at the warm temperature than are recovered when irradiation is carried out at the cold temperature. This effect becomes more pronounced as the dosage delivered to the flies is increased.

It is also evident from the figures presented in the last column of table 2 that, as the dosage is increased, on the average more chromosomes become involved in the interchanges. Therefore, the increase with dosage in the number of breaks per tested sperm is not solely to be attributed to an increase in the number of simple translocations involving only two chromosomes. The data of both the warm and cold temperature experiments indicate that an important factor which contributes to this increase is the parallel rise in the number of chromosomes involved in the interchanges.

ANALYSIS OF RESULTS

It is difficult to evaluate the significance of data such as these unless they can be compared with the results expected on the basis of some plausible theory of chromosome breakage and union. The general argument which follows forms the foundation for determining these expectations. For a given dosage of radiation, there is a probability, p(r), that a particular sperm will contain a total of r chromosome breaks in one or more of the K chromosomes being followed. These r breaks may be distributed among the K chromosomes in

various ways. For example, all r breaks may be in one chromosome or there may be two breaks in one chromosome, (r−2) in another and the remainder of the K chromosomes may not be broken. Therefore let p(d) be the probability that the r breaks will be distributed in a particular way. With each distribution of breaks there is a probability, $p(i_D)$, of detecting (by the genetic method of analysis) the interchanges formed by the union of the broken ends. Now the product $p(d) \cdot p(i_D)$ is the probability of detecting an interchange from a sperm whose r breaks are distributed in one of the possible ways. If this product is summed over all the possible distributions of r breaks among the broken chromosomes, the result is the probability, $S_D{}^r$, of obtaining a detectable interchange from a sperm with r breaks. Since p(r) is the probability that a sperm will have r breaks, then

$$\sum_{r=0}^{\infty} p(r) \cdot S_D{}^r$$

is the probability that for any particular dosage an interchange will be detected. From this figure it is possible to calculate the probability of securing a sperm with a translocation for any dosage if the relation between dosage and breakage is known. The reliability of the theoretical expectations depends on the proper evaluation of p(d), $p(i_D)$, and p(r); therefore, any experimental evidence pertaining to the assumptions made in the evaluation of these probabilities is of primary importance.

In order to determine p(d), it is necessary to know the relative probabilities that each of the chromosomes will be broken. It will be recalled that each of the major autosomes of *virilis* is about equally likely to be included in an interchange. This indicates that each of these chromosomes is equally likely to be broken. Therefore, the probability that there will be r_1 breaks in the second chromosome, r_2 in the third, r_3 in the fourth, and r_4 in the fifth is

$$\frac{r!}{r_1!r_2!r_3!r_4!}\left(\frac{1}{4}\right)^r,$$

where $r_1+r_2+r_3+r_4=r$. Since it is assumed that each of the major autosomes is equally likely to be broken, the above probability is also equal to the probability that there will be r_1 breaks in the third chromosome, r_2 in the fourth, r_3 in the fifth, and r_4 in the second, *etc.* Now if there are t *different* numbers of breaks per chromosome, s_1 of one number, s_2 of another number and s_t of the t^{th} number, then the number of possible arrangements of breaks having equal probabilities is the number of permutations of four things of which s_1 are of one type, s_2 of another type, *etc.* Accordingly, the desired probability that there will be r_1 breaks in one of the autosomes, r_2 in another, r_3 in another, and r_4 in the remaining one is

$$p(d) = \frac{4!}{s_1!s_2!\cdots s_t!}\,\frac{r!}{r_1!r_2!r_3!r_4!}\left(\frac{1}{4}\right)^r.$$

For example, if $r=7$, the probability that there will be three breaks in one chromosome, two breaks each in two other chromosomes, and no breaks in the remaining chromosome (3-2-2-0) is

$$\frac{4!}{1!2!1!}\frac{7!}{3!2!2!0!}\left(\frac{1}{4}\right)^7 = 0.15381.$$

It has been shown by BAUER (1942), FANO (1943), and HALDANE and LEA (1947) that if r breaks are formed in k out of the K chromosomes being followed, $2^{r-k}(r-1)!k$ viable arrangements of the broken ends are possible. The term "viable" means that the arrangement is eucentric and euploid. Discounting any induced dominant lethal mutations or any dominant sterility mutations, all of these arrangements should survive in the progeny of the test cross. The next step in the analysis, therefore, is to determine the number of these viable arrangements which are detectable as interchanges using the genetic method of analysis. Obviously, restoration of the original arrangement and any intrachromosomal rearrangements will not be observed. In a chromosome with one break, the only viable intrachromosomal arrangement possible is a restitution of the original arrangement; while in a chromosome with more than one break, the number of such viable arrangements of the broken ends of this chromosome is $2\cdot 4\cdot 6\cdots(2r_i-2)=(r_i-1)!2^{r_i-1}$. Thus the number of detectable interchanges is $2^{r-k}(r-1)!k-2^{r_1-1}(r_1-1)!\cdot 2^{r_2-1}(r_2-1)!\cdots 2^{r_k-1}(r_k-1)!$. If it is assumed that each of the viable arrangements is equally likely to occur, then the probability of obtaining an interchange in the test cross from a sperm with r breaks is obtained by dividing the number of detectable interchanges by the total number of viable arrangements. Upon simplification, this probability becomes

$$p(i_D) = 1 - \frac{(r_1-1)!(r_2-1)!\cdots(r_k-1)!}{k(r-1)!}.$$

The assumption that each of the viable arrangements is equally likely to occur implies not only that a broken end is just as likely to join with a broken end in another chromosome as it is to undergo restitution, but that the union of two or more broken ends in no way affects the joining of other ends except to reduce the number of ends which are available for union. This is a reasonable assumption made purely for the simplification of the mathematics.

In order to make a comparison with the empirical data presented on the minimum number of breaks per tested sperm, it is necessary to determine the expected portion of the detectable interchanges which involve two, three, and four chromosomes. Naturally if $k=0$ or 1, an interchange is impossible, and if $k=2$, all interchanges involve two chromosomes; that is, in the latter case the number of detectable interchanges is equal to the number of two-chromosome interchanges. In the case where $k=3$, the total number of viable interchanges involving only two chromosomes is the sum of the three possible products obtained by multiplying the number of detectable two-chromosome interchanges between any two chromosomes by the number of viable intra-

TABLE 3

Evaluation of theoretical probabilities.

NO. OF BREAKS r	POSSIBLE BREAK DISTRIBUTIONS	PROB. OF SUCH A DISTRIBUTION p(d)	PROB. OF A DETECTABLE INTERCHANGE $p(i_D)$	PROB. OF A 2 CHROMOSOME INTERCHANGE $p(i_2)$	PROB. OF A 3 CHROMOSOME INTERCHANGE $p(i_3)$	PROB. OF A 4 CHROMOSOME INTERCHANGE $p(i_4)$
2	2-0-0-0	.25000	—	—	—	—
	1-1-0-0	.75000	.50000	.50000	—	—
3	3-0-0-0	.11111	—	—	—	—
	1-1-1-0	.22222	.83333	.50000	.33333	—
	2-1-0-0	.66667	.75000	.75000	—	—
4	4-0-0-0	.01563	—	—	—	—
	1-1-1-1	.09375	.95833	.25000	.33333	.37500
	2-2-0-0	.14062	.91667	.91667	—	—
	3-1-0-0	.18750	.83333	.83333	—	—
	2-1-1-0	.56250	.94444	.38889	.55555	—
5	5-0-0-0	.00391	—	—	—	—
	4-1-0-0	.05859	.87500	.87500	—	—
	3-2-0-0	.11719	.95833	.95833	—	—
	3-1-1-0	.23437	.97222	.30555	.66667	—
	2-1-1-1	.23437	.98958	.12500	.33333	.53125
	2-2-1-0	.35156	.98611	.23611	.75000	—
6	6-0-0-0	.00098	—	—	—	—
	5-1-0-0	.01758	.90000	.90000	—	—
	3-3-0-0	.02930	.98333	.98333	—	—
	4-2-0-0	.04395	.97500	.97500	—	—
	4-1-1-0	.08789	.98333	.25000	.73333	—
	2-2-2-0	.08789	.99722	.09167	.90555	—
	3-1-1-1	.11719	.99583	.07500	.30833	.61250
	2-2-1-1	.26367	.99792	.05000	.26667	.68125
	3-2-1-0	.35156	.99444	.17222	.82222	—
7	7-0-0-0	.00024	—	—	—	—
	6-1-0-0	.00513	.91667	.91667	—	—
	5-2-0-0	.01538	.98333	.98333	—	—
	4-3-0-0	.02563	.99167	.99167	—	—
	5-1-1-0	.03076	.98889	.21111	.77778	—
	4-1-1-1	.05127	.99792	.05000	.27917	.66875
	3-3-1-0	.10254	.99815	.12778	.87037	—
	4-2-1-0	.15381	.99722	.13611	.86111	—
	2-2-2-1	.15381	.99965	.01458	.16944	.81563
	3-2-2-0	.15381	.99907	.05278	.94630	—
	3-2-1-1	.30762	.99931	.02778	.22917	.74236
8	8-0-0-0	.00006	—	—	—	—
	7-1-0-0	.00146	0.92857	.92857	—	—
	6-2-0-0	.00513	.98810	.98810	—	—
	4-4-0-0	.00641	.99643	.99643	—	—
	6-1-1-0	.01025	.99206	.18254	.80952	—
	5-3-0-0	.01025	.99524	.99524	—	—
	5-1-1-1	.02051	.99861	.03571	.25238	.71071

TABLE 3 (cont.)

NO. OF BREAKS r	POSSIBLE BREAK DISTRIBUTIONS	PROB. OF SUCH A DISTRIBUTION p(d)	PROB. OF A DETECTABLE INTERCHANGE $p(i_D)$	PROB. OF A 2 CHROMOSOME INTERCHANGE $p(i_2)$	PROB. OF A 3 CHROMOSOME INTERCHANGE $p(i_3)$	PROB. OF A 4 CHROMOSOME INTERCHANGE $p(i_4)$
	2-2-2-2	.03845	.99995	.00327	.06468	.93199
	5-2-1-0	.06152	.99841	.11270	.88571	—
	4-2-2-0	.07690	.99960	.03532	.96429	—
	4-3-1-0	.10254	.99921	.10397	.89524	—
	3-3-2-0	.10254	.99974	.02778	.97196	—
	3-3-1-1	.10254	.99980	.01587	.19603	.78790
	4-2-1-1	.15381	.99970	.01786	.20060	.78125
	3-2-2-1	.30762	.99990	.00675	.13611	.85704
9	9-0-0-0	.00002	—	—	—	—
	8-1-0-0	.00041	.93750	.93750	—	—
	7-2-0-0	.00165	.99107	.99107	—	—
	7-1-1-0	.00329	.99405	.16071	.83333	—
	6-3-0-0	.00385	.99702	.99702	—	—
	5-4-0-0	.00577	.99821	.99821	—	—
	6-1-1-1	.00769	.99926	.02679	.22917	.74330
	6-2-1-0	.02307	.99901	.09623	.90278	—
	3-3-3-0	.02563	.99993	.01171	.98823	—
	4-4-1-0	.02884	.99970	.08720	.91250	—
	5-2-2-0	.03461	.99980	.02560	.97421	—
	5-3-1-0	.04614	.99960	.08849	.91111	—
	5-2-1-1	.06921	.99985	.01250	.17798	.80937
	4-3-2-0	.11536	.99990	.01796	.98194	—
	4-3-1-1	.11536	.99993	.01071	.17292	.81629
	3-2-2-2	.11536	.99999	.00126	.04206	.95666
	4-2-2-1	.17303	.99996	.00379	.11548	.88069
	3-3-2-1	.23071	.99998	.00293	.11012	.88693
10	10-0-0-0	.00000	—	—	—	—
	9-1-0-0	.00011	.94444	.94444	—	—
	8-2-0-0	.00051	.99306	.99306	—	—
	8-1-1-0	.00103	.99537	.14352	.85185	—
	7-3-0-0	.00137	.99802	.99802	—	—
	5-5-0-0	.00144	.99921	.99921	—	—
	6-4-0-0	.00240	.99901	.99901	—	—
	7-1-1-1	.00275	.99950	.02083	.20932	.76934
	7-2-1-0	.00824	.99934	.08399	.91534	—
	6-2-2-0	.01442	.99989	.01951	.98038	—
	6-3-1-0	.01923	.99978	.07738	.92240	—
	6-2-1-1	.02884	.99992	.00901	.15972	.83118
	5-4-1-0	.02884	.99987	.07606	.92381	—
	4-4-2-0	.03605	.99997	.01181	.98816	—
	4-4-1-1	.03605	.99998	.00764	.15427	.83807
	4-3-3-0	.04807	.99998	.00655	.99343	—
	5-3-2-0	.05768	.99996	.01283	.98713	—
	5-3-1-1	.05768	.99997	.00787	.15496	.83714
	3-3-3-1	.06409	.99999	.00106	.09012	.90881
	4-2-2-2	.07210	1.00000	.00062	.03148	.96789
	5-2-2-1	.08652	.99998	.00238	.10053	.89707
	3-3-2-2	.14420	1.00000	.00045	.02589	.97367
	4-3-2-1	.28839	.99999	.00162	.09494	.90343

chromosomal arrangements possible in the third chromosome. The number of detectable interchanges including three chromosomes when $k=3$ is the total number of detectable interchanges minus the number of two-chromosome interchanges. Using similar considerations it is possible to determine the number of interchanges which involve two, three, or four chromosomes for any distribution of breaks among the four chromosomes being followed. Dividing these figures by the number of viable arrangements for the particular distribution of the r breaks, gives the probability of obtaining an interchange involving two chromosomes, $p(i_2)$, three chromosomes, $p(i_3)$, or four chromosomes, $p(i_4)$, where $p(i_D)=p(i_2)+p(i_3)+p(i_4)$. These probabilities along with p(d) for each of the possible break distributions have been calculated and are tabulated in table 3.

The probability, p(r), that for a given dosage a particular sperm will contain r breaks, remains to be evaluated. The Poisson distribution should fulfill the requirements if the breaks are produced individually and collectively at random over the range of dosage used. Since cytological difficulties prevent the observation of single breaks in Drosophila, the substantiating evidence for this assumption must come from other experimental organisms. CARLSON (1941) using the neuroblast cells of Chortophaga, and CATCHESIDE, LEA and THODAY (1946) studying structural changes in Tradescantia, found that the number of single breaks in a cell very closely follows the Poisson distribution. Thus if $\bar{r}$ is the average number of breaks produced by a given dosage, then

$$p(r) = \frac{\bar{r}^r e^{-\bar{r}}}{r!}.$$

This probability involves the assumption of some value for the average number of breaks per unit dosage. In this connection, it is to be noted that in the theoretical considerations so far it has been assumed that all broken ends eventually join. MULLER'S hypothesis (1940) that certain dominant lethal mutations may be caused by single chromosome breaks (the broken ends of the acentric and monocentric fragments fail to undergo union and, on subsequent division of the chromosome, the sister chromatids unite to form an acentric and a dicentric chromosome) has been tested further by PONTECORVO (1941, 1942). In these studies MULLER and PONTECORVO found that at low dosages the frequency of loss of single chromosomes increases linearly with the dosage administered. This provides indirect evidence that some of the dominant lethals are caused by single breaks which fail to unite. If all broken ends have an equal probability of remaining free and this probability is small, then the number of dominant lethals produced by the failure of one or more broken ends to unite should increase almost linearly with moderate dosages as do ordinary gene mutations. Therefore, the figure selected for the "average number of breaks" in the Poisson exponential is $\bar{r}$ multiplied by a coefficient, a, to take into account this particular type of dominant lethal and, in addition, dominant sterility mutations. The $a\bar{r}$ used was chosen such that at 1000 r units the theoretical and empirical values for the percent of sperm with inter-

changes are approximately equal ($a\bar{r} = 0.52$ per 1000 r units of X-radiation). There is some justification for this choice since the constitution of the interchanges induced by 1000 r units is comparatively simple (all of the 81 interchanges produced at this dosage involve only two chromosomes) and thus not subject to the complications invoked by numerous breaks. In addition, this is the point where the results of the warm and the cold temperature experiments nearly coincide.

TABLE 4

The theoretical dosage-frequency relation.

r	S_D^r	S_2^r	S_3^r	S_4^r	1000 r $\bar{ar}=0.52$ p(r)	2000 r $\bar{ar}=1.04$ p(r)	3000 r $\bar{ar}=1.56$ p(r)	4000 r $\bar{ar}=2.08$ p(r)
2	.37500	.37500	—	—	.08038	.19115	.25570	.27025
3	.68518	.61111	.07407	—	.01393	.06627	.13296	.18738
4	.90624	.52734	.34375	.03516	.00181	.01723	.05186	.09743
5	.97005	.34750	.49804	.12451	.00019	.00358	.01618	.04053
6	.99098	.20003	.53954	.25141	.00002	.00062	.00421	.01405
7	.99739	.10724	.50204	.38810	—	.00009	.00094	.00417
8	.99926	.05537	.42889	.51501	—	.00001	.00018	.00109
9	.99979	.02803	.34853	.62328	—	—	.00003	.00025
10	.99999	.01399	.27480	.71117	—	—	—	.00005
				$\sum_{r=2}^{10} S_D^r \cdot p(r) =$	**.04152**	**.13688**	**.25501**	**.37681**
				$\sum_{r=2}^{10} S_2^r \cdot p(r) =$	.03967	.12264	.21106	.28464
				$\sum_{r=2}^{10} S_3^r \cdot p(r) =$	.00175	.01299	.03857	.07780
				$\sum_{r=2}^{10} S_4^r \cdot p(r) =$	.00009	.00126	.00536	.01438
				$2\sum_{r=2}^{10} S_2^r \cdot p(r) + 3\sum_{r=2}^{10} S_3^r \cdot p(r) + 4\sum_{r=2}^{10} S_4^r \cdot p(r) =$	**.08495**	**.28929**	**.55927**	**.86020**

The final assumption to be made is that there is a linear relation between the dosage and the frequency with which breaks are formed. Although this linear relation can be inferred from many types of radiation studies on Drosophila (*e.g.*, BAUER 1942), the direct evidence must rest on the observation of single breaks. The experiments of CARLSON (1941) with Chortophaga and those of SAX (1940) and THODAY (1942) using Tradescantia show that such a relation actually exists. It has been assumed, therefore, that if the dosage is doubled $a\bar{r}$ is also doubled. This is not exactly true because as the dosage and thus $\bar{r}$ increase linearly, the coefficient a becomes smaller in approximately a linear fashion. Thus at the higher dosages the value of $a\bar{r}$ is somewhat overestimated.

There has been one assumption which has been implied but not stated in

the above considerations. This assumption is that no union of broken ends takes place during the period of treatment. If such union did occur, the empirical results would be distorted in relation to the theoretical expectations since the dosage administered to the flies was raised by lengthening the time of exposure rather than by increasing the amount of radiation per unit time. However, the evidence on this point for Drosophila seems quite clear. Neither MULLER (1940), DEMPSTER (1941), KAUFMANN (1941), nor MAKHIJANI (1945) was able to demonstrate any difference in the number of rearrangements formed when radiation was administered to sperm in such widely different ways as continuous treatment at different intensities, intermittent treatment, and continuous treatment with a delay of thirty days before mating. It is generally accepted that in Drosophila the union of broken ends takes place sometime during or after fertilization.

The final calculations leading to the theoretical dosage-frequency relation have been tabulated in table 4. It will be recalled that $S_D{}^r$, the probability of obtaining a detectable interchange from a sperm with r breaks, is obtained by summing the product $p(d) \cdot p(i_D)$ over all the possible distributions of the r breaks among the four chromosomes being followed. Similarly, $S_2{}^r$, $S_3{}^r$, and $S_4{}^r$ are the probabilities that an interchange involving two, three, or four chromosomes will be recovered from a sperm with r breaks. These figures are

TABLE 5

Comparison of the empirical and theoretical results.

APPROX. DOSAGE IN r UNITS		NO. OF SPERM WITH INTERCHANGES PER TESTED SPERM	MINIMUM NO. OF BREAKS PER TESTED SPERM	NO. OF OBSERVED INTERCHANGES	AV. MINIMUM NO. OF BREAKS PER INTERCHANGE	% INTERCHANGES INVOLVING 2, 3, AND 4 CHROMOSOMES		
						2	3	4
1000	W*	0.040	0.080	22	2.000	100.00	—	—
	C	0.042	0.084	47	2.000	100.00	—	—
	T	0.042	0.085	—	2.046	95.54	4.21	0.21
2000	W	0.097	0.203	50	2.100	90.00	10.00	—
	C	0.123	0.262	126	2.119	91.27	5.55	3.18
	T	0.137	0.289	—	2.113	89.60	9.49	0.92
3000	W	0.172	0.369	82	2.146	87.80	9.76	2.44
	C	0.256	0.562	218	2.197	82.57	15.14	2.29
	T	0.255	0.559	—	2.193	82.77	15.12	2.10
4000	C	0.353	0.805	163	2.282	74.85	22.09	3.07
	T	0.377	0.860	—	2.283	75.54	20.65	3.82

* W = warm temperature experiments, C = cold temperature experiments, T = theoretical expectation.

obtained by summing the product obtained by multiplying p(d) by $p(i_2)$, $p(i_3)$, and $p(i_4)$ respectively over all the possible distributions of the r breaks. These calculations have been carried out and the resulting probabilities are recorded in table 4. Calculations have not been made for values of r greater than 10 since there is less than one chance in 100,000 that a detectable interchange would not be recovered from a sperm with 10 breaks. The appropriate values of p(r), the Poisson distribution, for 1000, 2000, 3000, and 4000 r units

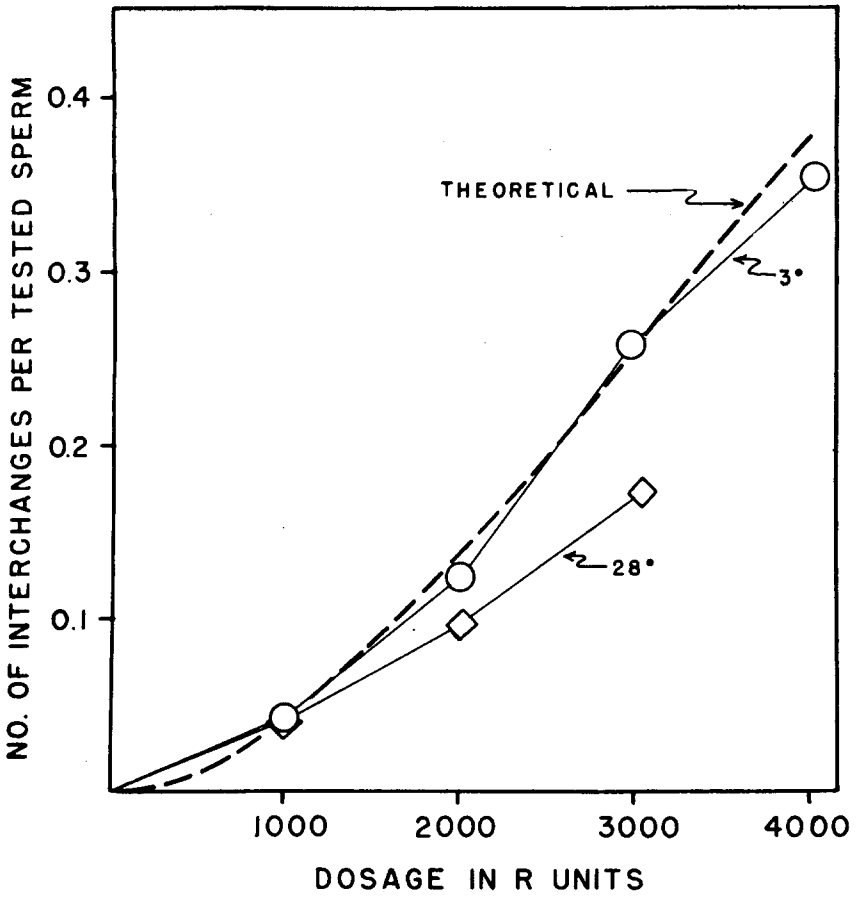

FIGURE 4. Comparison of the theoretical and empirical results. The dosage-frequency relation of the number of interchanges per tested sperm. See figure 2 for explanation of symbols.

are also tabulated in table 4. The final summations leading to the theoretical relation are presented at the bottom of this table. It will be noticed that the bold-faced figures in the first row of these summations give the probability of recovering a detectable interchange for the four dosage levels used in the experiments being reported. These values are actually the expected number of recovered interchanges per tested sperm. The summations in the next three rows of the table give the probability of obtaining an interchange involving

two, three, or four chromosomes for each of the four dosages used. The figures given in bold-faced type in the bottom row of this table are the expected minimum number of breaks per tested sperm since they are obtained by summing the product of the probability of securing a two, three, or four-chromosome interchange by the respective number of chromosomes involved.

The comparison between the theorectical expectations and the combined results of all experiments exclusive of any interchanges involving the Y chro-

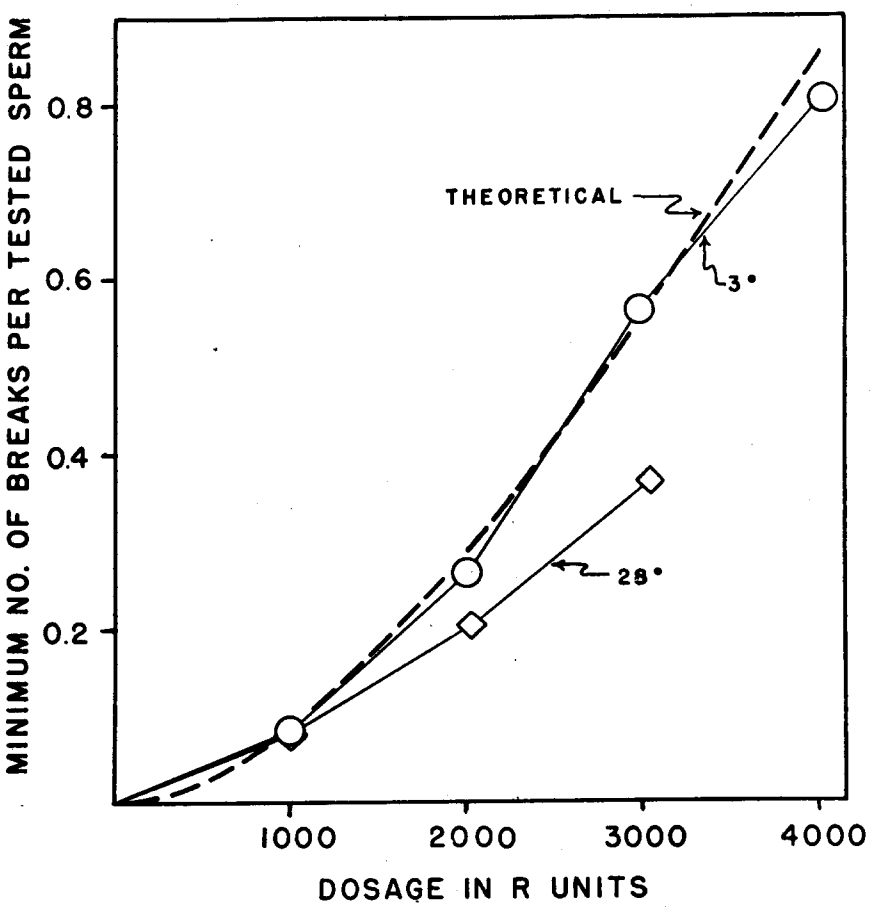

FIGURE 5. Comparison of the theoretical and empirical results. The dosage-frequency relation of the minimum number of breaks per tested sperm. See figure 2 for explanation of symbols.

mosome is made in table 5. The figures tabulated in the first two columns, relating to the number of sperm with interchanges and the minimum number of breaks, are presented graphically in figures 4 and 5 respectively. It will be noted that the results of the cold temperature experiments agree closely with the results expected from the theoretical considerations. This agreement is noteworthy since the theoretical curve probably represents a maximum curve because the assumption was made in its formulation that a broken end is as

likely to join with a broken end in another chromosome as it is to restitute. The question immediately arises as to whether this close fit is merely a coincidence or whether the chromosomes actually behave in accordance with the assumptions set forth in the theory. No definite answer can be given to this question; however, a clue is found by an examination of the relative frequencies with which translocations involving two, three, and four chromosomes are found. In the last three columns of table 5 are recorded the empirical and expected frequencies of occurrence of these three different types of interchanges. A chi-square test indicates that there is no significant difference between the expected relative frequencies and the actual values except in the case of the percent of four-chromosome interchanges in the cold temperature experiments conducted at 2000 r units ($\chi^2 = 4.8$). This close agreement provides some evidence that the assumption of random and independent union of broken ends is valid since the relative number of translocations involving three and four chromosomes should be reduced from the expectations if restitution is more likely to occur than interchange.

The second rather obvious conclusion to be drawn from the data presented in table 5 is that the results of the experiments at 28°C do not follow the expectations based on the theory. The decrease in the number of interchanges produced at the warm temperature is apparently not caused by any alteration in the randomness of the process of union since both the warm and cold temperature experiments closely follow the expected ratios with regards to the number of interchanges involving two, three, or four chromosomes. It is not surprising that a temperature change at the time of irradiation does not affect the joining of broken ends since this process takes place sometime during or after syngamy. Possible causes for the deviation of the warm temperature results will be discussed below.

THE FREQUENCY-DOSAGE RELATIONSHIP

The target theory of the biological action of ionizing radiations as applied to chromosome breakage has become a powerful working tool for present day investigators in this field. This theory implies that a single ionizing particle, either by one or more ionizations (hit) in the vicinity of particular regions of the chromosome thread (targets), is able to form a break in the chromosome. One expectation, based on the independent production of the individual particles, which follows immediately from the implications of the target theory is that the biological effect should increase linearly with the number of particles (dosage) until an appreciable number of targets have been hit. Although the determination of this expectation in Drosophila is aided by the fact that the biological effect, the breakage or potential breakage of the chromatin thread, undergoes no reverse change during the irradiation, the analysis is greatly hindered by the fact that the breaks themselves cannot be observed and thus the resulting chromosome arrangements must be used as the measure of the effect. A still further complication is introduced by the fact that only rearrangements of the chromatin are observable and in addition only those re-

arrangements which are viable in the heterozygous condition. From these considerations it is evident that the process of recombination of broken chromosome ends has of necessity been superimposed upon the series of events causing the breaks.

The lack of a complete understanding of this recombination process has made the interpretation of experimental results dealing with the relationship between dosage and the production of gross rearrangements most difficult. Although the work of OLIVER (1932), KHVOSTOVA and GAVRILOVA (1935), and CATCHESIDE (1938) were not in disagreement with a linear relation between dosage and gross chromosome exchanges, the extensive studies of BELGOVSKY (1937), KHVOSTOVA and GAVRILOVA (1938), MULLER (1938, 1940), TIMOFÉEFF-RESSOVSKY (1939), BAUER, DEMEREC, and KAUFMANN (1938), and BAUER (1939) clearly indicate that the number of rearrangements increases more rapidly than the first power of the dosage. On the basis of the latter results, MULLER (1938, 1940) proposed the "3/2 power rule". This rule means that gross rearrangements are produced in proportion to the 3/2 power of the dose. Although it is clear from numerous studies that the frequency with which these rearrangements are produced increases faster than the first power of the dose, the attachment of any great significance to the particular exponent of 1.5 seems rather dubious. It is obvious from the results presented in this investigation and from the findings of other workers, *e.g.*, BAUER *et al.* (1938), that at dosages of 4000 r units and above the slope of the frequency-dosage curve decreases giving the over-all curve a sigmoid appearance. Such a relation can hardly be described by a simple power function. Also it is apparent from the observed effect of temperature on interchange production that the exponent is a function of the temperature at the time of irradiation. And finally, it is evident from the mathematical analysis given that the particular exponent observed is related to the number of chromosomes being followed; that is, one would not expect to find the same exponent when translocations between only two chromosomes were being recovered as one would if four chromosomes were being studied.

The results of the present investigation are in accord with the experimental evidence on the dosage-frequency relation which has accumulated from the work with *melanogaster*. It is also evident that the cold temperature results are in agreement with the expectations based on the simple picture of the processes of chromosome breakage and union that is presented above. Two of the assumptions used in the formulation of this theory, namely, the random recombination of broken ends and the assumption that the union of two or more broken ends has no effect on the joining of the remaining ends, have been criticized by Fano (1941, 1943). The evidence upon which the first assumption is criticized is derived from analyses of the relative frequencies with which different types of rearrangements are recovered in the salivary gland chromosomes. Thus BAUER *et al.* (1938) and BAUER (1939) using *melanogaster*, HELFER (1941) and KOLLER and AHMED (1942) using *Drosophila pseudoobscura* found a wide discrepancy in certain cases between the recovered distribution of breaks among the chromosomes and the distribution "expected" on a

random basis. However, in determining the "expected" frequency used in these calculations no consideration is given to the possibility that restitution of breaks cause some sperm with a higher number of breaks to contribute to the class of rearrangements with fewer breaks. In spite of this omission in the calculations there may be a real discrepancy between the empirical and expected results.

Evidence that the second assumption is not wholly reliable comes indirectly with the discovery by KAUFMANN (1943) of a complex rearrangement involving at least 32 breaks. This was found in a larva whose father had received a treatment of 4000 r units of X-radiation and a subsequent exposure to infrared radiation. On the basis of the figure usually assumed for the average number of breaks per 1000 r units in Drosophila (0.4–0.6), the probability of obtaining a sperm with 32 or more breaks with 4000 r units of treatment is quite small. In addition, the probability (based on random union of broken ends) that such a sperm will give rise to a viable arrangement is about three percent (FANO 1943). Apparently FANO overcomes the obvious difficulty of explaining this case by assuming that the average number of breaks is higher than the figure stated above, and that restitution is much more likely to occur than exchange except when such an exchange happens to take place. That is, if an exchange has taken place during the process of union, this exchange imposes stresses along the chromosomes involved which cause any other breaks in these chromosomes to be less likely to restitute and thus become available for further unions. Although these suggestions appear reasonable, there is little evidence which contributes either to their support or rejection.

In view of the data just discussed, the question arises as to whether the rather close agreement between the results obtained in *virilis* and the theory can be attributed to the somewhat questionable procedure which is used in determining a value for the arbitrary constant ($a\bar{r}$) from the same experimental data to which the theoretical curve is being compared. It will be recalled that the value of $a\bar{r}$ was chosen such that only an agreement was reached at 1000 r units with regards to just the percentage of sperm with interchanges. However, with this choice it is found that the results of the cold temperature experiments agree at all dosage levels with the expectations in these three respects: (1) the percent of sperm with interchanges, (2) the minimum number of breaks per tested sperm, (3) the relative frequencies with which two, three, and four chromosomes are involved in the recovered interchanges. Furthermore, the value of $a\bar{r}$ that was chosen not only fits the results of these experiments but is in general agreement with the value postulated by other workers for the average number of breaks per 1000 r units in *melanogaster* (see LEA 1947). Thus it can be stated with some assuredness that the results of the experiments at the cold temperature are in concordance with the assumptions of random joining of broken ends and the independent union of these ends.

THE TEMPERATURE EFFECT

Soon after the discovery was made that gene mutations and chromosomal aberrations could be artificially induced, investigations were undertaken in

which the temperature of the organism receiving the radiation was altered during the period of treatment. The experimental designs which have been utilized to test for a temperature effect in Drosophila have been limited to two criteria: the rate of induction of sex-linked lethal mutations and the rate of translocation production as detected by genetic analysis.

MULLER's early experiments (1930) indicate that there is a slightly higher, but not significant, increase in the number of sex-linked lethal mutations induced in flies irradiated at 8° over a series treated at 34°C. MEDVEDEV (1935) found a significantly higher frequency of lethals produced in the flies maintained at 0° than in the ones treated at 20°C. However, TIMOFÉEFF-RESSOVSKY and ZIMMER (1939) could find no difference in the percentage of lethals induced at 10° and 35°C. This was confirmed by MULLER and MAKHIJANI (MULLER 1940) using temperatures of approximately 5° and 37°C. Finally, the recent work of KING (1947) substantiates MEDVEDEV's data since a two to three-fold increase in the mutation rate was observed in the series irradiated at 0° over the group maintained at a room temperature which fluctuated between 23° and 28°C.

The experiments which test the effect of differences in temperature on the production of translocations are apparently as contradictory as the results just cited. PAPALASHWILI (1935) found an increased number of translocations produced at 0°C over the number detected at room temperature. Similar results are reported in the more extensive tests of MICKEY (1939) who employed temperatures of about 4° and 28°C and KANELLIS (1946) who irradiated the flies at 2° and 32°C. But MULLER and MAKHIJANI (*op. cit.*) using the same temperatures as in the mutation studies, could find no increase in the number of interchanges in the cold series. It has been reported by KING (1947) that MAKHIJANI in 1944 was unable to observe a temperature effect on the production of chromosome aberrations. (The writer has not seen this paper and consequently the temperatures at which the radiation was given are not known.)

It is difficult to reconcile all these findings. MULLER (1940) has attributed the detection of a positive temperature effect to poor experimentation caused by the failure to keep the radiation from secondary radiators the same in the cold and warm series and to the inclusion in the results of sperm which had not completed the maturation divisions at the time of treatment. In certain of the experiments cited care was not taken to control these factors; however, the results reported in this paper cannot be subjected to this criticism. There is one consistent factor present in the Drosophila experiments which has in the past been overlooked. Apparently the assumption has been made that the wider the temperature range employed, the more pronounced any effect should be. This is not necessarily the case. It seems more than a passing coincidence that in all of the experiments reporting a positive effect of temperature, the cold temperature used varied from 0° to 4°C and the warm temperature from 20° to 32°C. While in all of the experiments indicating no effect, the two temperature ranges used varied from 5° to 10°C and from 34°

to 37°C. It appears, therefore, that an increase in the induction rate of translocations accompanies a change in temperature either above or below room temperature. This increase is similar to the result PLOUGH (1917) obtained while studying the effect of temperature on the frequency of crossing over in *melanogaster*. He found that at 13° and 31°C the percent of crossing over is almost three times greater than at 27°C. In order to prove that such an increase actually occurs, the mutation and interchange rate should be measured in comparable experiments conducted at 28° and 36°C.

The question now arises as to whether temperature affects the breakage or the joining process. In Drosophila sperm no union of broken chromosome ends takes place until syngamy, thus these two processes are separated in time. Although it is difficult to see how a temperature change at the time of irradiation could affect the course of union at fertilization, it is equally perplexing to see how it could alter the process of chromosome breakage since the physical action of X-rays is independent of temperature in the range utilized (LEA 1947). The union of broken ends is not delayed in Tradescantia and it has been postulated that the decrease in the number of chromosomal aberrations observed when the temperature is raised during X-radiation is caused by an alteration in the process of union (SAX and ENZMANN 1939; FABERGÉ 1940; CATCHESIDE, LEA and THODAY 1946; and SAX 1947). The recent work of FABERGÉ (1948) casts doubt on this interpretation since he found a temperature effect on the number of aberrations when the radiation and temperature change were administered in about one-third of the average time that a break remains free. When viewed in this light, it is not surprising that MULLER (1940) and KAUFMANN (1946b) were unable to show an effect of different temperatures on Drosophila females fertilized by irradiated sperm; that is, temperature differences at the time of union. FABERGÉ also reports that a change in temperature in either direction immediately preceding treatment considerably increased the number of aberrations. He states that a possible explanation of this behavior may lie in the fact that thermal diffusion caused by the temperature gradient moves the chromosome threads, thus making restitution less likely. It is tempting to draw an analogy to the Drosophila experiments which apparently show a rise in the frequency of translocations at temperatures above and below room temperature; however, in many of these experiments the flies were placed in the cold chamber a sufficient length of time before treatment to neutralize any temperature gradient.

The possibility also exists that the temperature effect is not caused by the temperature *per se* but rather by its effect on some cellular process such as respiration. Recently, THODAY and REED (1947) demonstrated a three-fold increase in the number of anaphases showing chromosome aberrations when oxygen instead of nitrogen was bubbled for a short time before and after X-ray treatment through a cell of water containing *Vicia faba* roots. Cytological examinations were made at intervals after treatment varying from a few to 48 hours. The fact that the increase was consistently evident at these various times would seem to indicate that the breakage, not the union process

was being affected. If the activity of a fly at a particular temperature is taken as a rough index of the rate of respiration, the work of CHADWICK (1939) on the frequency of wing beat of Drosophila would indicate that the respiration rate is greatest at a temperature of about 29°, being much lower at 8° and 36°C. Thus at 29°C there would presumably exist the greatest gradient in oxygen tension within the tissues and consequently a lowering of the oxygen concentration in the testis. Viewed in this light, the temperature effect in Drosophila falls into line with the bean root experiments; however, the mechanism whereby the oxygen tension affects chromosome breakage is unknown.

It is evident from the considerations discussed above that, although the method whereby heat at the time of X-ray treatment affects chromosome breakage is still undetermined, there seems no reason to doubt that the temperature effect on irradiated Drosophila chromosomes is a reality. The evidence obtained from the *virilis* experiments indicates that the decrease at the warm temperature in the number of sperm with interchanges is not associated with the process of union of the broken chromosome ends, since the relative number of translocations involving two, three, and four chromosomes agrees with the cold temperature results and with the expectation based on the assumption of random and independent union. It seems most probable that a temperature difference at the time of irradiation alters, directly or indirectly, the process of chromosome breakage.

SUMMARY

Males of *Drosophila virilis* were treated with 1000, 2000, 3000, and 4000 r units of X-radiation. During irradiation the flies were kept at a temperature of either $3 \pm 1°$ or $28 \pm 1°$C in comparable experiments. Any major chromosome interchanges induced in the sperm of these males involving the four main autosomes and the Y chromosome were recovered by genetic analysis of their F_2 offspring.

The relationship between dosage and translocation production as determined by these experiments is found to be similar to the relationship previously reported in *D. melanogaster*. The percent of sperm with interchanges increases more rapidly than the first power of the dose, but gradually slopes off around 4000 r units giving a sigmoid-shaped curve. A somewhat similar relation is found to exist between dosage and the minimum number of breaks induced in the sperm. This increase in the number of breaks is not caused solely by a rise in the number of simple translocations recovered, but is in part caused by the fact that, per individual sperm, more chromosomes are involved in the interchanges induced at the higher dosages.

The frequency-dosage relation is not the same in the warm and the cold temperature series. A significant lowering of the rate of induced interchanges is observed at the higher dosage levels when the flies are maintained at the warm temperature during treatment. This finding confirms the results of other investigators who used warm temperatures of around 25°C and observed a temperature effect. It has no direct bearing on the negative results reported by workers who employed a warm temperature of approximately 36°C.

An analysis of the chromosomes involved in over 800 of the induced trans-

locations indicates that the four major autosomes are about equally likely to participate in any interchange, but the frequency with which the Y chromosome is involved is less than half as great as any one of the autosomes. This indicates that the four major autosomes are about equally likely to be broken by the ionizing radiations.

In order to evaluate the significance of the observed frequency-dosage relation, the mathematical expectations based on a simple theory of chromosome breakage and union are derived. The formulation of this theory involves the following main assumptions:

1. The number of breaks per sperm follows the Poisson distribution.
2. The mean number of breaks per sperm increases linearly with dosage.
3. Each of the major autosomes is equally likely to be broken.
4. The broken chromosome ends unite at random to form the resulting arrangements.

A comparison between the theoretical expectations and the empirical results (exclusive of interchanges involving the Y chromosome) brings to light several pertinent facts concerning the processes of chromosome breakage and union in *D. virilis*. The cold temperature experiments follow very closely at all dosage levels the expectations not only in respect to the percent of sperm with interchanges, but also in regards to the minimum number of recovered breaks. Moreover, a close agreement is evident between the expected and actual frequencies with which interchanges involving two, three, and four chromosomes occur. On the basis of this result it is apparent that restitution of broken chromosome ends is no more likely to occur than recombination, thus lending support to the assumption that the union process is a random and independent joining of broken ends. The results of the warm temperature experiments fall below the expectations based on the theory. This decrease in the number of recovered interchanges cannot be attributed to a disturbance in the process of joining of the chromosome ends which would increase the chance of restitution, since the relative frequencies with which two, three, and four chromosomes are involved in an interchange again agree with the expectation based on the assumption of random union. Although it is not known whether the temperature effect is caused by a direct action of heat on the chromosomes being irradiated, it seems likely that the ultimate effect of heat alters the mechanisms involved in the breakage of the chromosome thread.

ACKNOWLEDGEMENT

The writer is deeply appreciative of the counsel and encouragement given him by PROFESSORS J. T. PATTERSON and W. S. STONE. The writer is also indebted to the NATIONAL RESEARCH COUNCIL for granting him a PREDOCTORAL FELLOWSHIP during the course of this investigation.

LITERATURE CITED

BAUER, H., 1939 Röntgenauslösung von Chromosomenmutationen bei *Drosophila melanogaster*. I. Bruchhäufigkeit, -verteilung und -rekombination nach Speicheldrüsenuntersuchung. Chromosoma **1**: 343–390.

1942 Röntgenauslösung von Chromosomenmutationen bei *Drosophila melanogaster*. II.

Die Häufigkeit des primären Bruchereignisses nach Untersuchungen am Ring-X-Chromosom. Chromosoma **2**: 407–458.

BAUER, H., M. DEMEREC, and B. P. KAUFMANN, 1938 X-ray induced chromosomal alterations in *Drosophila melanogaster*. Genetics **23**: 610–630.

BELGOVSKY, M. L., 1937 Dependence of translocation frequency in *Drosophila melanogaster* upon X-ray dosage. Trud. Inst. Genet. **11**: 93–124.

CARLSON, J. G., 1941 An analysis of X-ray induced single breaks in neuroblast chromosomes of the grasshopper (*Chortophaga viridifasciata*). Proc. nat. Acad. Sci. **27**: 42–47.

CATCHESIDE, D. G., 1938 The effect of X-ray dosage upon the frequency of induced structural changes in the chromosomes of *Drosophila melanogaster*. J. Genet. **36**: 307–320.

CATCHESIDE, D. G., D. E. LEA, and J. M. THODAY, 1946 The production of chromosome structural changes in Tradescantia microspores in relation to dosage, intensity and temperature. J. Genet. **47**: 137–149.

CHADWICK, L. E., 1939 Some factors which affect the rate of movement of the wings in Drosophila. Physiol. Zool. **12**: 151–159.

DEMEREC, M., and B. P. KAUFMANN, 1941 Time required for Drosophila males to exhaust the supply of mature sperm. Amer. Nat. **75**: 366–379.

DEMPSTER, E. R., 1941 Absence of a time factor in the production of translocations in Drosophila sperm by X-irradiation. Amer. Nat. **75**: 184–187.

FABERGÉ, A. C., 1940 An experiment on chromosome fragmentation in Tradescantia by X-rays. J. Genet. **39**: 229–248.

1948 Action of temperature during X-ray treatment of Tradescantia chromosomes. Genetics **33**: 104.

FANO, U., 1941 On the analysis and interpretation of chromosomal changes in Drosophila. Cold Spring Harbor Symp. Quant. Biol. **9**: 113–120.

1943 Mechanisms of induction of gross chromosomal rearrangements in Drosophila sperm. Proc. nat. Acad. Sci. **29**: 12–18.

HALDANE, J. B. S., and D. E. LEA, 1947 A mathematical theory of chromosomal rearrangements. J. Genet. **48**: 1–10.

HELFER, R. G., 1941 A comparison of X-ray induced and naturally occurring chromosomal variations in *Drosophila pseudoobscura*. Genetics **26**: 1–22.

KANELLIS, A., 1946 Abhängigkeit der Rate strahleninduzierter Chromosomenmutationen bei *Drosophila melanogaster* von der Temperatur während der Bestrahlung. Naturwiss. **33**: 27.

KAUFMANN, B. P., 1941 The time interval between X-radiation of sperm of Drosophila and chromosome recombination. Proc. nat. Acad. Sci. **27**: 18–24.

1943 A complex induced rearrangement of Drosophila chromosomes and its bearing on the problem of chromosome recombination. Proc. nat. Acad. Sci. **29**: 8–18.

1946a Organization of the chromosome. I. Break distribution and chromosome recombination in *Drosophila melanogaster*. J. Exp. Zool. **102**: 293–320.

1946b Modification of the frequency of chromosomal rearrangements induced by X-rays in Drosophila. III. Effects of supplementary treatment at the time of chromosome recombination. Genetics **31**: 449–453.

KHVOSTOVA, V. V., and A. A. GAVRILOVA, 1935 A new method of detecting translocations. Biol. Zh. **4**: 905–916.

1938 Relation between the number of translocations in *Drosophila melanogaster* and the X-ray dosage. Biol. Zh. **7**: 381–390.

KING, E. D., 1947 The effect of low temperature upon the frequency of X-ray induced mutations. Genetics **32**: 161–164.

KOLLER, P. C., and I. A. R. S. AHMED, 1942 X-ray induced structural changes in the chromosomes of *Drosophila pseudoobscura*. J. Genet. **44**: 53–72.

LEA, D. E., 1947 Actions of radiations on living cells. xii and 402 pp. Cambridge Univ. Press and The Macmillan Co.

MAKHIJANI, J. K., 1945 Mutation frequency as conditioned by the manner of application of radiation. Indian Jour. Genet. and Pl. Breeding **5**: 15–31.

MEDVEDEV, N. N., 1935 The contributory effect of cold with irradiation in the production of mutations. Compt. Rend. Acad. Sci. U. R. S. S. **4:** 283–285.

MICKEY, G. H., 1939 The influence of low temperature on the frequency of translocations produced by X-rays in *Drosophila melanogaster*. Genetica **21:** 386–407.

MULLER, H. J., 1930 Radiation and genetics. Amer. Nat. **64:** 220–251.

1938 The remaking of chromosomes. Collect. Net Woods Hole **13:** 181–195, 198.

1940 An analysis of the process of structural change in chromosomes of Drosophila. J. Genet. **40:** 1–66.

OLIVER, C. P., 1932 An analysis of the effect of varying the duration of X-ray treatment upon the frequency of mutations. Z.I.A.V. **61:** 447–488.

PAPALASHWILI, G., 1935 The effect of a combined action of X-rays and low temperature on the frequency of translocations in *Drosophila melanogaster*. Biol. Zh. **4:** 587–591.

PLOUGH, H. H., 1917 The effect of temperature on crossingover in Drosophila. J. Exp. Zool. **24:** 147–209.

PONTECORVO, G., 1941 Induction of chromosome losses in Drosophila sperm and their linear dependence on dosages of radiation. J. Genet. **41:** 195–215.

1942 The problem of dominant lethals. J. Genet. **43:** 295–300.

SAX, K., 1940 An analysis of X-ray induced chromosomal aberrations in Tradescantia. Genetics **25:** 41–68.

1947 Temperature effect on X-ray induced chromosome aberrations. Genetics **32:** 75–78.

SAX, K., and E. V. ENZMANN, 1939 The effect of temperature on the frequency of X-ray induced chromosome aberrations. Proc. nat. Acad. Sci. **25:** 397–405.

THODAY, J. M., 1942 The effect of ionizing radiations on the chromosomes of *Tradescantia bracteata*. A comparison between neutrons and X-rays. J. Genet. **43:** 189–210.

THODAY, J. M., and J. REED, 1947 Effect of oxygen and the frequency of chromosome aberrations produced by X-rays. Nature **160:** 608.

TIMOFÉEFF-RESSOVSKY, N. W., 1939 Zur Frage der Beziehungen zwischen strahlenausgelösten Punkt- und Chromosomenmutationen bei Drosophila. Chromosoma **1:** 310–316.

TIMOFÉEFF-RESSOVSKY, N. W., and K. G. ZIMMER, 1939 Mutationsauslösung durch Röntgenbestrahlung unter verschiedener Temperatur bei *Drosophila melanogaster*. Biol. Zbl. **59:** 358–362.