THE FREQUENCY OF TRANSLOCATIONS PRODUCED BY X-RAYS IN DROSOPHILA

H. J. MULLER
University of Texas, Austin, Texas

AND

EDGAR ALTENBURG
Rice Institute, Houston, Texas

Received September 13, 1929

TABLE OF CONTENTS

By the term "translocation" is meant the breaking off of a piece of one chromosome and its attachment to another, non-homologous chromosome. An isolated case of a translocation was encountered in 1918 by BRIDGES (1923). Although involving too small a portion of chromatin to be noticed cytologically by the means available, it was thoroughly demonstrated by genetic means. This case stood alone, however, as a genetic oddity, unparalleled by similar cases that could be satisfactorily proved, until 1926. In that year STERN (1926, 1927) discovered a case in which a portion of the Y chromosome had become attached to the X; this was demonstrated both genetically and cytologically. Somewhat later in 1926, and in 1927, MULLER independently obtained genetic evidence that in his X-ray experiments numerous changes in gene alignment, of varied kinds, including translocations, had been produced (MULLER 1927a); some of these cases too were checked cytologically in 1927 (MULLER 1927b, 1928a, PAINTER and MULLER 1929). It then became of interest to discover with what frequency such translocations between non-homologous chromosomes might arise, as a result of exposure to short wave-length radiation, and of what different types these translocations might be.

GENETICS 15: 283 Jl 1930

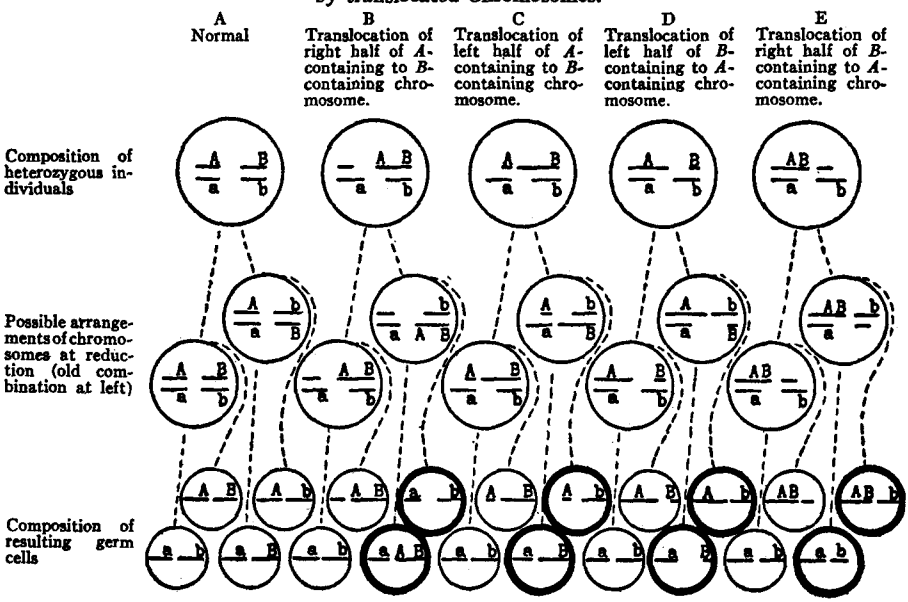
THE METHOD OF ATTACK

In order to investigate this question systematically, crosses were then started of irradiated males by non-radiated females which differed from the males in a number of "identifying genes" or "markers," located in different chromosomes, and some hundreds of the heterozygous offspring (F_1) were then backcrossed, in individual cultures, so that the distribution of the markers among the progeny of each of them could be determined. Ordinarily, the character differences dependent on different pairs of chromosomes would be distributed at random, according to Mendel's law of "independent segregation," but, if a translocation had occurred, the markers in the chromosomes involved would now appear as though linked.

By way of illustration, suppose that in one pair of chromosomes of the heterozygous F_1 which is to be tested there are the allelomorphic markers A and a, and in another pair B and b, so that the F_1 individual has, so far as these are concerned, a composition which can be represented by $\frac{A}{a}\ \frac{B}{b}$, (showing homologous chromosomes by parallel lines of different thickness, one below the other). Then, in the normal course of inheritance, the chromosomes might, of course, become arranged at reduction with equal likelihood either in the "old combination" $\frac{A}{a}\ \frac{B}{b}$, giving germ cells with $\underline{\quad A}\ \underline{\quad B}$ and with $\underline{\quad a}\ \underline{\quad b}$, respectively, or in the "new combination," $\frac{A}{a}\ \frac{b}{B}$, giving germ cells with $\underline{\quad A}\ \underline{\quad b}$ and with $\underline{\quad a}\ \underline{\quad B}$, respectively (see figure 1, diagram A). But if there had been a translocation in the sperm cell from which the F_1 had been derived, breaking the A-containing chromosome and tying the piece of it bearing A to the B-containing chromosome, the F_1 composition would be represented by $\frac{\quad A}{a}\ \frac{B}{b}$, and the chromosomes at reduction could have either the "old" arrangement $\frac{\quad}{a}\ \frac{A}{\quad}\ \frac{B}{b}$ or the "new" arrangement $\frac{\quad}{a}\ \frac{\quad}{A}\ \frac{b}{B}$. (If crossing over were possible still other arrangements could occur.) The first arrangement would yield the old combinations of markers $\underline{\quad A}\ \underline{\quad B}$ and $\underline{\quad a}\ \underline{\quad b}$, respectively, but the second arrangement would yield germ cells of composition $\underline{\quad}\ \underline{\quad b}$ and $\underline{\quad a}\ \underline{A}\ \underline{\quad B}$, respectively. The former of these new-combination germ cells (namely $\underline{\quad}\ \underline{\quad b}$) and the progeny

resulting from it obviously have a deficiency of the genes in the right half of the originally *A*-containing chromosome, while the latter of the two new combinations carries an excess of genes of this same region. It is therefore likely that one or both of these new combinations will give abnormal and relatively inviable zygotes, or, in the case of a relatively large or potent translocation, that the new combinations will both be fully lethal.[1] Thus, so far as the non-crossover offspring are concerned, at any rate, only the old combinations of markers, *AB* and *ab*, may be found alive among the F_2, the combinations *Ab* and *aB* being absent, just as though *A* and *B* had been completely linked, or only the old combinations and one new-combination class will appear, or the new combinations may simply be less numerous and show phaenotypic abnormalities.

Figure 1.—Diagram showing nature of combinations produced by normal and by translocated Chromosomes.



(The germ cells in heavy outline contain combinations apt to be lethal to zygotes formed on backcrossing.)

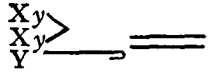
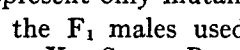
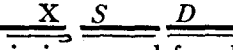
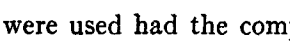
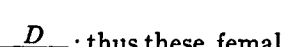
The same result would be just as apt to occur if the "left" end of the left hand chromosome had been attached to the right hand chromosome,

[1] It had been repeatedly insisted upon by MULLER in the earlier Drosophila work that a change from the normal proportions ("balance") of genes, caused by an excess or deficiency in the numbers of some genes in relation to the numbers of the others, would tend to produce phaenotypic abnormalities, including inviability. This conception is now fully proved by the present results even as regards disproportions involving the excess or deficiency of *parts* of chromosomes.

as follows: $\frac{A}{a} - \frac{B}{b}$. For, even though A and B are not physically connected here, nevertheless, just as before, the two "new" non-crossover combinations, receiving one of these markers and not the other, will have a deficiency and an excess, respectively, of genes, and will hence tend not to be normal or viable. This result would also follow if either end of the B-containing chromosome should become attached to the A-containing chromosome, or, for that matter, if either the a- or the b-containing chromosome should become broken and have a fragment attached to the other. The failure of one or both new combination classes (Ab or aB) to appear would therefore indicate a translocation between the non-homologous chromosomes containing the markers studied but would not by itself tell us which chromosome was broken and attached to the other nor which end of the broken chromosome had become attached. In some such cases it might even happen, theoretically, that both chromosomes (A-containing and B-containing) had become broken, mutually exchanging parts—a condition recently recognized by BLAKESLEE and BELLING in Datura under the term "segmental interchange," but these cases too would give results like the others, in the above tests. The objective of such tests, therefore, is to determine the total frequency of all kinds of translocations between the "marked" chromosomes which disturb the "genic proportions" sufficiently to cause the inviability, or the abnormality, of one or both of the new combinations.

THE INITIAL RESULTS—A STARTLINGLY HIGH FREQUENCY OF TRANSLOCATION

In the first experiment (fall of 1927), the X-rayed males carried in one of their second chromosomes the dominant gene for curly wings (C_y), in the other second chromosome the dominant star eye (S), and in one of their third chromosomes the dominant dichaete (D), thus having the composition $\frac{X}{Y}\ \frac{C_y}{S}\ \frac{D}{\ }$ (representing the sex chromosomes, the second pair and the third, in this order, but in the case of the autosomes, labelling only the mutant allelomorphs). They were given the treatment which has been designated as "t4" (MULLER 1928b) and immediately afterwards mated to non-rayed females having the normal allelomorphs of the above genes but carrying attached X-chromosomes with the genes for yellow body, and a supernumerary Y chromosome. The composition of the females might therefore be represented

as follows  . The offspring from this cross which carried the father's X and the mother's Y would be males; those carrying the father's Y and the mother's attached X's would be females. Half the offspring would carry C_y, the rest would carry S. Half would carry D, and the rest its normal allelomorph, but only the D-carrying offspring were used, since a marker in the third chromosome was needed. If, now, we show the chromosomes derived from the rayed father by heavy lines, and again represent only mutant genes in the autosomes, the composition of half of the F_1 males used was  , and of the other half  . Males of both these types were then crossed with virgin normal females, one male to a culture, and the distribution of sex, curly or star, and dichaete, with regard to each other, among the progeny ("F_2") in each of the 90 fertile cultures was examined, to discover any cases in which any of the expected new combinations of the characters in question failed to appear, or appeared in very small numbers. F_1 females were also bred, one to a culture, by normal males. The females which were used had the composition  and  ; thus these females also might reveal translocations, through counts of the distribution of sex, curly or star, and dichaete, with regard to each other.

It will be seen that the tests of the F_1 males were adapted to reveal translocations involving the rayed X chromosome and the second or third, while the tests of the females would reveal translocations involving the rayed Y chromosome and the second or third. In the case of the Y, however, only cases in which an autosome had been broken and attached to the Y would be discoverable by these methods, not the converse cases of breakage of the Y and its attachment to an autosome, since it is known that excess or deficiency of the Y chromosome or parts of it usually produce no change in viability or appearance.

It should be noted also, concerning the tests of the F_1 females, that D does not serve as an absolute marker for the whole of the third chromosome as it does in the male, since crossing over can occur between it and other parts of this chromosome, in the female. Hence, in the tests of females, some cases of translocations involving the third chromosome may have gone unrecognized, and other cases might be recognizable only by the lessened frequencies of the "new combination" classes rather than by their

complete absence, inasmuch as crossing over might produce viable, genically balanced classes phenotypically simulating the inviable new chromosome-combinations that carry an excess or deficiency of genes. Similarly, crossing over might occur between star and a point of breakage or attachment in the second chromosome. Curly, however, formed a quite satisfactory marker for the whole of the second chromosome, where it was used, since it is associated with an unusual gene-arrangement that undergoes very little crossing over with a normal second chromosome. The close agreement of the results from the female tests with those from the male tests indicates, in general, that few of the translocations were missed in the former which would have been found in the latter.

The numbers of translocations found in this first experiment, and the chromosomes involved, are summarized in table 1, lines 1a and 1b, for the male and female tests separately. In all there were 21 translocations found among the 161 F_1 flies (representing as many X-rayed sperm cells of P_1 tested), or 13 percent. This was a startlingly high frequency, in view of the previous uniqueness of the phenomenon, and was comparable with the rate of detectable gene mutations occurring after X-raying. If the natural translocation rate is similarly high, compared with the natural rate of gene mutation, it must be of widespread, even though *relatively* infrequent, occurrence in nature and may be of importance in evolution.

Examining the sorts of translocations, it is seen that all the possible combinations of the chromosomes studied together were found: namely X-II, X-III, Y-II, Y-III, and II-III(where X-II means a translocation from either X to II or II to X; similarly for X-III, Y-II, etc.). The last class, II-III, was the most numerous, as might perhaps have been expected in consideration of the fact that it involved exclusively the two largest chromosomes. The relative frequencies of the different kinds of translocations will be considered in more detail later, when the results of all the experiments have been presented.

The counts from the individual cases of translocation are shown in table 2. Translocations involving II and III are evidenced by an absence or decided deficiency in numbers of the C_y or S non-D and the non-C_y or non-S D recombination classes. Translocations involving the Y or X and an autosome give results as though either C_y or S or D, as the case might be, had become sex-linked. Thus, if a piece of the second chromosome had become attached to the Y, then the F_1 males, on being bred to normal females, would transmit this piece to their sons, but not to their daughters. The curly (or star) daughters would therefore fail to

develop, since in them the second chromosome would lack the piece, and since the Y carrying the piece attached would also be absent. But in the curly male offspring this piece missing from the second chromosome would be attached to the Y chromosome and they could live. Hence, no curly daughters develop, but curly sons do. The non-curly sons, however, have the piece in triple amount, since one "dose" of it is attached to the Y, and two additional doses are present in their normal positions in the second chromosome pair, one of these normal chromosomes having been the untreated chromosome of the F_1 male, the other having come from the normal mother. On account of the genic disproportion thus produced, the non-curly males would fail to develop. The non-curly females, on the other hand, would not have the piece in triple amount (but simply in double amount) since they receive their father's X (not his Y, with the extra piece). Hence they live. In other words, in the case of a translocation of an autosome to the Y, all, or the great majority, of the flies of one sex show one of the autosomal traits involved, while the flies of the opposite sex show the allelomorph. Similar reasoning applies to an X-II translocation.

As expected, the counts from the tested F_1 females are not as clear-cut as from the males (on account of crossing over). From one of the females (case 15) a batch of progeny was derived in which a double translocation was evident, involving II, III and Y at the same time; here, since II and III gave the same results as though they were tied together, it was not possible to determine whether the translocation to the Y was from II or from III. (This accounts for the caption "sex and II or III" in table 1 at the head of the seventh column).

An additional kind of test was carried out in the case of the cultures derived from the tested F_1 females of this experiment. Here, owing to the method of inheritance imposed by the attached X's the F_2 males carried the Y chromosome that had been present in the rayed sperm of the original P_1 male, and that had been transmitted through the F_1 female of the composition that has been represented by $\overset{Y\rightharpoonup}{X_v X_v}\rangle$ If this Y chromosome had had a piece broken off by the X-rays, which had become lost or which had become translocated to a rayed autosome, then, although the F_1 female herself should be fertile(the Y seems to affect the female very little), nevertheless she should give rise to sons (F_2) inheriting the deficient Y, and, as STERN has shown, such males may be sterile. In the case of a complete loss of a fragment of the Y, all the sons (F_2) would be sterile; in the case of a translocation, all the sons receiving the non-radiated autosomes,

and therefore failing to receive the translocated fragment, would be sterile. All the males of the latter class (so far as they could be determined by the markers) were therefore tested, in mass culture, by normal females, in the case of each F_1-F_2 culture derived from a tested F_1 female, but no instances were found in which all males of the given class proved sterile. Nevertheless, direct tests of individual F_1 males from rayed P_1 fathers crossed by normal (not attached-X) females, showed considerable sterility, as MULLER had previously reported for his experiments. The above tests would hence indicate that most of this sterility in the F_1 males is not due to breakage of the Y chromosome.

CONFIRMATION OF THE FINDINGS, AND DEMONSTRATION OF THEIR CONNECTION WITH THE TREATMENT

A second translocation experiment was then undertaken, involving a cross of X-rayed (t4) S/C_y males by females having the combination of recessive genes in chromosome III termed "IIIpl" (roughoid hairy scarlet pink spineless ebony) The use of the multiple stock here, as well as in the following experiments, had no advantage over the C_y (or S) D stock in getting the translocation frequencies, but was used with a further purpose in mind The F_1 males in this second experiment carried the radiated Y Their composition may be represented as $\frac{Y}{X}\ \frac{S \text{ or } C_y}{\overline{\text{IIIpl}}}$. These F_1 males were then used as P_2, being backcrossed, individually, to females carrying IIIpl or some part of IIIpl (in the combination a_r p_x, s_p "theca" referred to below) and the cultures were examined for the presence of recombinations among the progeny (F_2). As table 1, line 2 shows, 13 translocations of type II-III were found, and 3 involving the Y, in a count of 110. In this case, although the dosage factors were so far as possible the same as before, a new machine was used, which later tests have shown to give only a half to a third the effective output, when the controllable factors (time, distance, voltage, amperage, filter and surroundings) are sensibly the same as before. The counts from the translocations are shown in table 2, cases 21-36. In this instance, for some unexplained reason, the total translocation frequency is, if anything, even higher than before, but the disparity between the number of II-III's and those involving the sex chromosome is still evident.

In the second experiment 84 of the 110 P_2-F_2 cultures were kept track of in such a way that the recency of origin of the translocations found in them could be proved or disproved, that is, the fact that these transloca-

tions had not or had been present in the stock previous to the generation in which irradiation was carried out. This inquiry was undertaken as a substitute for the breeding of controls. The desired end was accomplished simply by recording the P_2-F_2 cultures in groups, any given group of cultures being "brother-cultures," in the sense that the P_2 males in all the cultures of this group were brothers, having been derived from the same treated P_1 male, and, in fact, carrying a derivative of the same second chromosome of that P_1 male (all cultures of the given group carrying C_y or all carrying S). If, now, a given translocation, recognized from the F_2 count of one of these cultures, had really been present in the original P_1 male before treatment (having been received by him from one of his parents), it would have been transmitted by him to all of his progeny (the F_1 or "P_2") of the type used as P_2 in the group. The finding of a certain translocation (Y-II, Y-III, or II-III) in one culture of the group and not in another is therefore tantamount to the finding that this translocation did not arise in any generation antecedent to the P_1 male. Two fertile cultures in a group are enough to prove this point, and the conclusion would still be valid even if both of them contained translocations, if it could be shown that they did not contain the same translocation. The records of these cultures, by groups, are given in table 3.

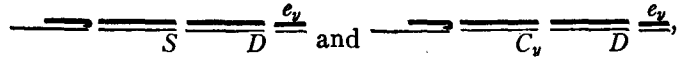
It will be seen that all of the 12 translocations found in these 84 cultures originated in what would be called the generation of the P_1 male. It is evident that this rate of origination must be far higher than what occurs in untreated material. For while little or no work has previously been done to determine the frequency of occurrence of translocations in control material, still many individual crosses involving genes in separate chromosomes have of course been carried out on Drosophila, and if translocations of natural origin occurred at all frequently, a fair number of natural cases should already have been found in the Drosophila investigations. If the 12 translocations here in question could have been regarded as an accumulated store, inherited from many previous generations, it might possibly have been contended that they might have been of "natural" origin, but when it is recognized that they must all have originated in one generation (the treated generation) it becomes obvious that the natural frequency of translocation cannot possibly be high enough to account for this, and that nearly all, or all of them, must have been due to treatment. This being the case it was considered unnecessary, for the purpose of the subsequent experiments, to run control tests in them or to keep track of the grouping of the P_2-F_2 cultures.

In a third experiment, radiated male flies having recessive genes in both the second and third chromosomes (in the second: arc, plexus, and speck; in the third: thread, stripe, sooty, rough, and claret, a combination termed "theca") were crossed to yellow females of the race with attached X's. The F_1 males, here carrying the radiated X, were tested, by backcrossing them to virgin females having the second and third chromosome recessives named. In the present experiment the new X-ray machine was used, and the factors of treatment would lead to a calculated dose of "t8" (about equivalent to the old "t3"). The numbers and kinds of translocations are given in table 1, line 3. It will be seen that the results are very similar to those of the first experiment. One of the translocations here too was a double one, involving II and III, and an attachment between the X and either II or III (which one being indeterminate from these counts). The individual counts from the cases of translocation are given in table 2, cases 37 to 55.

EXPERIMENTS INVOLVING THE FOURTH CHROMOSOME

A series of experiments (4 to 7) was next undertaken which had as one of its objects the ascertainment of the frequency of detectable translocations involving the small fourth chromosome, as compared with those involving other chromosomes. The recessive mutant gene termed "eyeless" (e_y) versus its normal allelomorph was used as the marker for the fourth chromosome throughout these experiments, while the markers for chromosomes II and III remained *S* or C_y, and *D*, as in experiment 1. In experiments 4 to 6, however, all the dominants were brought into the P_2 flies from their non-radiated parents, while in experiment 7 the reverse was the case. The dosage was "t8" in experiments 4, 6, and 7, and "t12" in experiment 5, the new machine being used in each case.

The P_1 cross in experiments 4 to 6 was of irradiated "eyeless" males to females carrying Star (*S*) in one of their second chromosomes and Curly (C_y) in the other, and carrying *D* in one of their third chromosomes and in the other "$C_{III}x$" (a lethal suppressor of crossing over produced by a previous raying). The F_1 males chosen for breeding were those which had received *D*. These were therefore of the following composition:



again representing the irradiated chromosomes with heavy lines. These F_1 males were then used as P_2, by backcrossing them, one male to a culture, to virgin females having all recessive genes at the loci in question (namely, non-*S*, non-C_y, non-*D*, eyeless). The offspring (F_2) in each P_2-F_2 culture

were then examined in order to detect the absence of classes having recombinations of the markers in question (considered two at a time). It will be observed that the Y, II, III and IV were subject to study. The experience previously gained in the detection of translocations was now found to be sufficient to enable their detection to be effected without the making of counts, except in special cases.

In experiment 7 the irradiated P_1 flies were males of composition $\frac{(Y)}{(X)}$ $\frac{ST}{}$ $\frac{D}{}$ (only the mutant genes being represented, as usual.) Here *T* represents the gene for truncate wings, which was introduced with an ulterior object in view. So far as the determination of translocations is concerned, *T* may be disregarded here, since it has little expression in the heterozygous condition unless intensifiers are present in other loci; these were not present in this experiment. The P_1 females were homozygous for e_v, but otherwise normal. The F_1 flies which were used for breeding, as P_2, were males having the composition $\frac{}{} \frac{ST}{} \frac{D}{e_v}$ (again representing the treated chromosomes with heavy lines). As in experiments 4 to 6, they were backcrossed in individual cultures to recessive females (normal except for eyeless) and recombinations of all the markers, two at a time, were looked for.

The results of each of these experiments are summarized in table 1, lines 4 to 7. In all, 7 translocations involving the fourth chromosome were found, in a count of 420; all of these have been confirmed by later tests. It is practically certain, on theoretical grounds, that all of these cases were transfers from the large autosomes to the fourth. For not only is it probable that breakages of the fourth chromosomes would be very infrequent, as compared with breakages of the larger chromosomes, but, if they did occur, and were accompanied by an attachment of one of the fragments to a non-homologous chromosome, the recombinations carrying only such a small excess or deficiency of chromatin would almost surely be viable, in view of the known fact that individuals with an entire fourth chromosome extra or missing are also viable and not greatly different in appearance from the normal (the "triplo-IV's" and "haplo-IV's" of BRIDGES); hence such cases would probably not have been found by the methods used. Later analysis of the translocations in question has borne out this inference.

Curiously enough, two of the seven translocations to the fourth chromosome occurred in cases of double translocations; that is, in both these

cases two simultaneous translocations took place in the same treated sperm cell of the P_1 male. One of these cases was in experiment 5, where there was a simultaneous translocation between II and III, and to IV from either II or III, it being indeterminable which of the latter had its fragment attached to the fourth, inasmuch as all viable offspring that inherited the radiated II also inherited the radiated III. (This is why this translocation to the fourth had to be recorded as "II or III and IV.") The count of the F_2 cultures showed, instead of 16 classes in approximately equal numbers, only the following: $8 C_v\ D$ ♀, $5\ C_v\ D$♂, $5 e_v$ ♀, $6\ e_v$♂, 4 "normal" ♀. The "normals" were evidently triplo-IV individuals, caused by non-disjunction of the fourth chromosomes. Such non-disjunction might be expected, theoretically, owing to the large translocated autosomal fragment, through its affinities for its normally placed homologue, interfering with the synapsis and disjunction that should occur between the fourth chromosome to which it is attached and the normal fourth chromosome. The other case was in experiment 4, where the same P_2-F_2 culture showed both the II-IV translocation and also the Y-III translocation recorded in that experiment. The count in this case gave, instead of 16 classes: $12\ S\ D$ ♀, $12\ D\ e_v$♀, $11\ S$ ♂, $5\ e_v$♂. Later tests showed some non-disjunction of chromosome IV to occur in this case also.

Flies inheriting both the Y-III and II-IV translocations which arose in the case last described were then subjected to another irradiation, in order to produce a II-III translocation between these chromosomes, so that a stock giving no recombinations whatever of genes present in the father might be obtained. This end was readily accomplished, one of the desired translocations being found in a batch of 25 P_2-F_2 cultures from a second irradiation with a t11 dose. Males of the resultant Y-II-III-IV translocation stock, then, always transmit this entire set of chromosomes to their fertile male offspring (though some sterile non-disjunctional males also occur), and these offspring show a similar genetic behavior, as do all fertile descendants in the direct male line. The female offspring, on the other hand, except where non-disjunctional, must always receive the entire homologous set of chromosomes that their father contained, just as though the chromosomes of each haploid set had been joined into a single chain.

In experiments 4 to 7 care was taken not to overlook cases in which one recombination class was absent while the contrary recombination class was present in the F_2 cultures. Three such cases involving II and III were found (1 each in experiments 4, 5, and 6), and they have been included

among the II-III translocations in table 1. In the case in experiment 4 the class (C_y) containing the non-radiated second and the radiated third chromosome was viable; in the cases in experiments 5 and 6, on the other hand, it was the opposite recombination which was the viable one. There is reason, based on analysis of other cases, to believe that when only one of two contrary recombination classes derived from Drosophila translocations is viable, that class is usually the "hyperploid," the one carrying the overdose of certain genes, while the lethal recombination is the class having the deficiency of genes, that is, the "hypoploid." If this is true, then the first of the translocations just referred to involved a transfer from II to III, while the other two involved transfers from III to II.

COMPARATIVE FREQUENCIES OF DIFFERENT TRANSLOCATIONS

We may now take count of the comparative frequencies of the translocations involving different chromosome combinations. In experiments 4 to 7 there were 7 translocations from II or III to IV and 8 or 9 translocations from II or III to Y (transfers in the opposite direction being excluded in the case of both these kinds of translocations, as previously explained). It therefore seems as though fragments of the long autosomes were about as likely to become attached to the small fourth as to the Y chromosome. When, however, we examine the results of the other experiments involving the Y, we find a somewhat higher frequency than in experiments 4-7 of the Y-II and Y-III translocations, when the latter are compared with the frequency of II-III translocations as a standard of reference. It may be, therefore, that the observed frequency of Y-II and Y-III translocations in experiments 4-7 was the result of a chance fluctuation that caused it to be somewhat below the mean value. Since the II-IV and III-IV frequencies would not necessarily fluctuate in the same direction it is possible that the translocations to the Y are really somewhat more numerous than to chromosome IV; this difference in frequency, if it exists at all, cannot be very great, however, and certainly is not proportionate to the difference which exists between the physical sizes of these chromosomes, as cytologically observed.

In order to compare the frequencies of translocations involving the Y and the X, respectively, we may again find each frequency in terms of its relation to the frequency of II-III translocations found in the same experiment, using the latter as our standard of reference. From table 1, on the line marked "Total for X, II, III," we find that there were 8 translocations of types X-II plus X-III, as compared with 18 of types II-III, that is, a ratio

of 1:2.2. On the line below this we find that there were 17 (or 18) translocations of types Y-II plus Y-III, as compared with 67 of type II-III, a ratio of 1:3.7. Thus these figures indicate a higher (apparently nearly twice as high) incidence of translocations involving the X than of translocations involving the Y. The absolute number (8) of X translocations found is, however, too small for us to be certain of the "significance" of such a difference in frequency.

Such a difference as above indicated would be expected if the translocations of types "X-II" and "X-III" found include translocations in both directions (from II to X and also from X to II, etc.). For, as previously stated, the translocations found involving Y were always in the same direction—from the autosome to the Y, those from the Y to the autosome having remained undetected. However, there is evidence that a somewhat similar limitation applies also in the case of the X, when the present methods of detection are employed. For, as we shall see later, breeding tests have indicated that a translocation is usually accompanied by the appearance of a recessive lethal in the chromosome which underwent breakage. Thus a broken X, received by an F_1 male, would usually cause the death of the latter, even though both fragments were present. It was the F_1 males, (derived from crosses of radiated P_1 males by attached-X females) which were used (experiments 1a and 3) in tests of translocations involving the X chromosome. Hence classes "X-II" and "X-III" probably represent almost exclusively translocations in the direction "II to X" and "III to X." The three translocations involving the X which appeared in experiment 1a were analyzed genetically, and all of them proved to be in this direction. Translocations in the opposite direction can occur, for in other experiments of MULLER, in which females containing irradiated X's were tested, translocations in the direction, from the X to the autosome, have been found; these in most, but not all, cases have proved lethal in the male.

If, then, we consider our present classes "X-II" and "X-III" as being in only one direction, the difference in frequency between these and the Y translocations appears more pronounced, and the difference in frequency between the translocations involving the X and those involving IV cannot be due to "fluctuations of sampling" (8 translocations to X in an experiment yielding only 18 of type II-III, and 7 translocations to IV in an experiment yielding 48 of type II-III). The results will, however, be less equivocal in an experiment in which all types can be studied at once, since there is some indication that the frequencies of different types of translocations, relative to one another, may also be subject to variation due to determinate causes.

Regardless of our conclusions concerning the frequencies of the translocations to X, to Y, and to IV, respectively, relatively to each other, there is no room for doubt that the frequency of each of them is very significantly lower than the frequency of type II-III. Since class "II-III" is in reality composed of the translocations from II to III plus those from III to II, it is legitimate to compare its frequency with that of the sum of X-II and X-III or the sum of Y-II and Y-III, or the sum of II-IV and III-IV, inasmuch as any single one of these other classes comprises translocations exclusively, or nearly exclusively, in one direction, namely, from the larger to the smaller chromosome, being in this respect unlike II-III. As table 1 shows, there were, in experiments yielding 18 of class II-III, 8 of classes X-II plus X-III; there were, in experiments yielding 67 of class II-III, 17 or 18 of classes Y-II plus Y-III; and there were, in experiments yielding 48 of class II-III, 7 of classes II-IV plus III-IV. These give ratios of 1:0.45, 1:0.27, and 1:0.15, respectively. In brief, then, the figures 1:0.45:0.27:0.15 represent the relative likelihoods, as indicated by our present data, of a fragment from a long autosome becoming attached to the other long autosome, to the X, to the Y, or to the fourth chromosome, respectively.

The cause of this variation in the likelihood of attachment to the different chromosomes is still obscure. Light may be shed upon it through more detailed analysis of the structures of the individual translocations; this is a project now well under way. If, for example, a considerable proportion of the translocations were mutual, involving breakage of and transfer from and to both chromosomes concerned at once (BLAKESLEE'S "segmental interchange"), it would follow that (so far as these mutual translocations were concerned) their frequencies might be roughly proportional to the products of the lengths of the two chromosomes taking part (supposing that the chance of breakage in a chromosome is roughly proportional to its length). As yet, however, the evidence seems against most of the translocations in Drosophila being mutual. Again, we might account to some extent for the apparent relation between the length of a chromosome and the likelihood of its serving for the attachment of a fragment from another if the attachment point could be anywhere along its length, rather than always terminal. This would not necessarily require the fragment to become attached to the side of a chromosome—a process that might lead to difficulties in its growth and longitudinal division. It might also come about if the "recipient" chromosomes sometimes underwent a breakage simultaneous with that of the "donor" chromosome, accompanied by reunion of the pieces in a different arrangement from that of before ("inversion" or "in-

sertion"), one or both of the breakage points thus serving for the attachment of the fragment from the donor.

Neither mutual translocation nor any kind of mutual breakage, nor side attachment, can well be the rule, however, else we should expect a much greater disparity between the different classes of translocations than actually exists. For the figures 1.0:0.45:0.27:0.15 by no means express the relative lengths of the chromosomes respectively serving as recipients; these are more nearly given by the figures 1.0:0.67:0.67:0.01. The products of the lengths show still greater disparities. In various cases already analyzed, moreover, it has been proved that the recipient chromosome underwent no breakage or rearrangement, and that the attachment was terminal, though mutual translocations have also been found.

Still other problems would be raised if it should be found that the relative frequencies of the different kinds of translocations differ in different experiments. For example, a comparison of the figures for experiments 5 and 6, in table 1, suggests that in the former there were significantly fewer translocations to Y and IV, in proportion to the II-III translocations, than there were in the latter. If such differences were to be substantiated, we could no longer use the II-III class as a fixed standard of reference, and our relative figures would have meaning only when considered in connection with certain other determining conditions in the experiment, of a nature at present unknown.

THE EFFECT OF TRANSLOCATIONS WHEN HOMOZYGOUS

Reference has been made in the foregoing to the finding that the majority of the translocations were lethal when homozygous. Experiments 3 to 6, inclusive, were purposely framed in such a way that no lethals (for example, C_v, S or D, which are all lethal when homozygous) would be in the radiated chromosomes to begin with; these therefore would not prevent the obtainment of the translocated chromosomes in homozygous condition. Various specimens of translocations, of different kinds, involving X, Y, II, III, and IV, have now been sampled, in tests constructed for the purpose of getting them homozygous. Relatively few could be obtained homozygous, and those that could have usually shown sterility and different kinds of morphological abnormalities, when homozygous, just as though they contained a gene-mutation or as though, like the hyperploid and hypoploid types, they were genically disproportioned. The detailed evidence on these points must be reserved for another paper.

Three possible explanations present themselves for this at first sight surprising situation.

(1.) There has been a simultaneous gene mutation (these are usually

lethals) at some other locus in the chromosome, at a distance from the locus of breakage or attachment. Such a coincidence, occurring in the majority of cases, seems highly unlikely. However, there is some as yet inconclusive experimental evidence that one genetic change tends to be associated with another elsewhere in the same cell, so we can not abruptly dismiss this possibility as yet.

(2.) The alteration in intermolecular surroundings of the genes directly adjacent to the points of breakage and reattachment, in other words the alteration in intergenic contiguities, has in itself brought about a change in the quantity or quality of the physico-chemical action of these genes upon the protoplasm, so as to make them, in effect, somewhat different genes, as though gene-mutations had taken place in the genes on either side of the breakage and attachment points. Plausibility is lent to such an assumption through STURTEVANT'S finding that two genes for bar eye adjacent to one another in the same chromosome seem to have an amount of effect on the development of the eye different from that of two otherwise identical genes for bar eye that lie in separate, homologous chromosomes. Fundamental problems of genetics are involved here which merit active prosecution.

At present we have at least one ray of light on the point here at issue. If this assumption is true, then the genes next to the attachment point on the recipient chromosome should become changed in their action just as much as the genes next to the breakage point on the donor chromosome. Nevertheless, in the cases where there was the possibility of distinguishing such an effect—the cases of translocation *to* the X, 3 or 4 of which have been studied in some detail, and in which the male serves to reveal any changes in genes of the X—no lethal action or visible abnormalities could be detected. It is true that these may only count as one case in point, since the attachment was in each instance to the right hand end of the X and it might be assumed that this was for some reason a locus particularly insensitive to the sort of change in question. However, till further evidence is forthcoming,* these cases must be taken as opposed to the present interpretation.

(3.) There is finally the possibility that in the act of breakage a chromosome usually becomes injured, so that one or more of the genes next to the breakage point either become destroyed, or are caused to mutate in some way. (Not that they are made functionally different by their changed relationships, as in possibility 2, in which case a reestablishment of the original alignment would automatically restore the original genotype

* More light on the question will be obtained when a study is made of the effect of attaching extra segments to the end of X chromosomes which have, through previous gene rearrangement, acquired new terminal points.

exactly, but that they have been permanently altered by some "injurious" process which accompanied the breakage, but which was not inseparable from the latter.) By a process of elimination, this possibility seems for the time being the most probable. It agrees in general with BRIDGES' interpretation of why his original translocation was not viable when homozygous—namely that the attached piece did not represent quite all of the originally detached piece, a small portion next to the breakage point having become lost in the process of breakage or transfer. It is not, however, necessary to suppose that the small portion was lost; it may merely have been caused to mutate. A breakage, if it could occur in the middle of a gene, instead of between genes, would be bound to cause such a loss or at least mutation; this possibility merges into that of alternative 2. If the genetic material be regarded as a continuum, instead of as segmentally arranged in units, the genes, there would be no distinction between an intra-genic and an inter-genic break, and all breaks would partake of the nature of gene-mutations, as postulated in 2. Reasons have, however, been given (MULLER 1926–1929) for regarding the genetic material as segmental.

The above being the apparent possibilities, they must be kept in mind so that as the evidence concerning translocations accumulates it may be considered constructively, in its bearing on these questions, and also so that new methods of experimental attack on them may if possible be evolved. But no matter which of the possible causes, if any, should turn out to be correct, one apparent effect of the phenomenon in question is so obtrusive that it cannot be ignored here. That is, since most translocations are found to be lethal when homozygous, and the remainder seem commonly to be sterile and abnormal, it would at first sight seem highly unlikely that translocations could play a role in the evolutionary processes occurring in nature. For the establishment of the ordinary translocation in homozygous condition, in a group of individuals, would be tantamount to exterminating the group.

The situation is, however, not so impossible as it might seem at first sight. If the translocations are accompanied by gene mutations, or by genetic changes similar in their effect to gene mutations, it is only to be expected that the majority of them all would be lethal, and that, of the remainder, which were not lethal, the majority would be antagonistic to survival and reproduction. For, as is well known, that is true of the gene mutations themselves, and it is just what is to be expected of any random changes occurring in a complex organization. Nevertheless, evolution must have proceeded through gene mutations, that is, through the very rare gene mutations which happened not to be detrimental, and which there-

fore could withstand the test of natural selection. The numerous lethals, which perished, were thus only a necessary concomitant of the same process which produced the relatively few mutations of a non-detrimental character. The same may therefore be true also of translocations and other changes in gene alignment. In fact, the somatic or physiological changes commonly accompanying translocations may from this point of view be considered as an aid to their becoming established in a species, for then there might be a few adaptive translocations which would have the aid of natural selection in spreading through a group of the species. On the other hand, if the translocations never had any somatic expression their spread would occur only under extraordinary accidental circumstances, as through a series of successive decimations of a population, in each of which only a few chance-chosen individuals survived; undoubtedly, however, such events happen more than once in most groups, in the course of thousands of generations.

THE FREQUENCY OF TRANSLOCATIONS AS COMPARED WITH THAT OF GENE MUTATIONS.

Although the survival of translocations, provided they occur, thus presents no insuperable obstacles, the question of their frequency of occurrence remains to be considered. If their frequency is far below that of ordinary gene mutations we should expect far fewer translocations than gene mutations to become incorporated in a stock in the course of its evolution during a given period. Evidence derived from a comparison of the cytology, and of the genetic maps, of related species of Drosophila, indicate this to be almost certainly true [see the work of METZ (1914, 1916) and of STURTEVANT 1921, STURTEVANT and PLUNKET 1926], though *some* changes in gene alignment, both inter- and intra-chromosomal, must undoubtedly have occurred since the origination of the genus. Direct experimental evidence on the present question is lacking, since no systematic searches for translocations in control material, similar to the mutation frequency studies of the authors, have been made. If, however, many of the mutations in somatic characters which have been observed in untreated material had been accompaniments of translocations or of other gene rearrangements, considerable positive evidence of this should, it seems, already have been adduced in all the locus determinations which have been made of the Drosophila mutations. And such evidence has not so far appeared.

From the present studies, on the other hand, we may obtain some direct evidence regarding the frequency of translocations induced by X-rays, as compared with gene mutations similarly induced. In all the data here

presented, together, there was a total of 117 translocations found, each of which involved at least one breakage of one of the long autosomes, and its attachment to some non-homologous chromosome or other in the same cell. There would probably have been about 8 more such translocations found, if chromosome IV had been studied in all the experiments, thus making a more complete figure of 125. These were distributed among a total of 883 tested F_1-F_2 cultures, from P_1 males given an average X-ray dosage of "t9" (in terms of the newer machine). The frequency of translocations from both of the long autosomes was therefore 125/883 or 14 percent. Thus we must conclude that a given one of the long autosomes, subjected in the sperm cells to treatments of about t9 strength, becomes broken and translocated to some non-homologous chromosome—X, Y, IV, or the other long autosome—in approximately 7 percent of the treated sperm cells. (Correction is not made here for mutuals or for possible differences in frequency to X and to Y.)

This figure of 7 percent for a "t9" dose may then be compared with the figure 12 percent, which represents the number of detectable "mutations"—mainly lethals—found by MULLER in 1926 and 1927 in the X-chromosome of sperm cells given the equivalent of a "t12" dose ("t4" on the old machine) (MULLER 1928b), or with the figure 8 percent, the percentage of X-chromosome lethals found by HARRIS this year (HARRIS 1929), following a "t8" (new machine) treatment of the sperm. It must, however, be remembered that the long autosomes are about one and a half times as large as the X, both cytologically and genetically. In a given portion of a long autosome about the size of the X, then, we should find only about $2/3 \times 7$ percent, or 4.7 percent of breakages accompanied by translocations, with a t9 dose, or, if the effect is proportional to dosage, about 0.5 percent with a t1 dose. This, then, is to be compared with 1.0[2] percent of visible plus lethal "mutations" in the X-chromosome per each "t1" of dosage. The rate of translocations detectable by the present methods in the space of a "unit" in one of the long autosomes is therefore just about half the rate of "mutations" detectable by the methods used for these, in about the same space on the X-chromosome. These "mutations," however, themselves included a considerable proportion of chromosome abnormalities. It is likely, moreover, that at least half of the translocations which occurred escaped detection. This follows from the fact that any particular fragmentation breaks the chromosome into a fiber-bearing fragment (the longer fragment, in the case of the long centrally attached autosomes), and a fragment without fiber attachment. If now the fiber-bearing fragment of a chromosome became attached to another

[2] Oliver's recent work shows 1.25 percent to be the more nearly correct figure here.

chromosome, an abnormal chromosome, having two points of fiber attachment, would become formed, while the other fragment, lacking any fiber, would fail to be transported in cell division, and an inviable offspring would probably ensue. The real rate of occurrence of translocations may therefore be just as high as, or higher than, that of gene mutations, after X-ray treatment has been applied to the sperm cells.

This conclusion is rather surprising, if, as now seems likely from the work of HANSON (1929a) and of OLIVER (1930), the genetic effectiveness of X-rays remains proportional to dosage, and if in addition natural mutations and translocations should be mainly due to "natural X-rays." For in that case we should expect as many natural translocations to occur as natural detectable "point mutations." We can be virtually sure that this is not the case. Owing to the property which translocations have of being usually accompanied by lethal or other detectable phaenotypic effects, a large number of them should have been found in the work of discovery and analysis of apparent gene mutations in Drosophila, if the translocations were really comparable in numbers to the gene mutations, even though translocations as such had not been specifically looked for. The absence of cases in the earlier work thus indicates a real scarcity of them, as compared with the frequency of ordinary gene mutations. There can, however, be no doubt about the high frequency of the translocations artificially induced in the present experiments, and the fact that this frequency must have been comparable with that of the gene mutations here. How then shall we reconcile these two conflicting series of findings?

It must be remembered that the natural cases dealt with represent a sum total of the mutations that have occurred at all different stages of the life cycle, and in either sex, indiscriminately. All the induced translocations and gene mutations considered, on the other hand, occurred at one restricted stage—namely, in the mature spermatozoa. There is some evidence, from work of HANSON (1929b) and of HARRIS (1929), that the germ plasm at this stage is peculiar in its sensitivity to radiation. At least, considerably more of both gene mutations and chromosome abnormalities occur in offspring from cells radiated in the spermatozoan stage than in those from other rayed cells; conceivably, however, this may be due to a higher selective death rate of the mutant cells in stages other than that of the spermatozoa. Whether the gene-mutation frequency or the chromosome-abnormality frequency is raised more has not as yet been determined. However, work of MULLER (as yet unpublished) on the relative

TABLE 1

NUMBER OF EXPERIMENT	X-RAY MACHINE USED	X-RAY DOSAGE	SEX OF F_1 TESTED	TOTAL FERTILE CULTURES	NUMBERS OF TRANSLOCATIONS INVOLVING: SEX AND II	SEX AND II OR III	SEX AND III	II AND III	SEX AND IV	II AND IV	II OR III AND IV	III AND IV	CHROMOSOMES STUDIED
1	old	t4{a	♂	90	2		1	6					X II III
		t4{b	♀	71	3	1	2	6					Y II III
2	new	t4	♂	110			3	13					Y II III
3	"	t8	♂	192	2	1	2	12					X II III
4	"	t8	♂	44			1	4		1			Y II III IV
5	"	t12	♂	172	(1?)		1	23			1		Y II III IV
6	"	t8	♂	159	3		1	15		3		2	Y II III IV
7	"	t8	♂	45	2			6					Y II III IV
Total for X, II, III (1a; 3)				282	4	1	3	18					X II III
Total for Y, II, III (1b, 2, 4, 5, 6, 7)				601	8(+1?)	1	8	67					Y II III
Total for II, III, IV (4, 5, 6, 7)				420				48		4	1	2	II III IV
Entire Total				883	12(+1?)	2	11	85	0	4	1	2	(X or Y) II III (IV).

Among the cultures in experiment 5 were 2 in which all the offspring (F_2) contained the nonradiated second chromosome, bearing Curly. There were 28 offspring in each of these cultures. Evidently a dominant lethal had been induced in chromosome II; the viability of the P_2 male containing it may have been due to its not having contained the lethal in all tissues of its body (owing to the fractional effect often shown by mutants in the first generation after radiation of the sperm) or it may have been due to an incompleteness of action of the lethal (the latter then being really a "semi-lethal"). One clear case of a dominant semilethal induced in chromosome II, that produced a disarrangement of the bristles, was also encountered in experiment 5, and one dominant semilethal, producing various thoracic abnormalities, was found associated with a II-III translocation yielding a count of 21 C_y D and 2 non-C_y non-D (variants).

A "delta"-like vein abnormality, located in the radiated third chromosome, was found in one of the cultures of experiment 6 not involving a translocation.

TABLE 2

Progeny counts in F_2 cultures involving translocations and other abnormalities.

EXPERIMENT NUMBER	CASE NUMBER	SEX OF F_1 TESTED	SEX CHROMOSOMES TESTED	MARKERS USED IN		NUMBERS OF FLIES RECEIVING RADIATED CHROMOSOMES AS INDICATED BY MARKERS (S_x = *radiated* X OR Y)								TYPE OF ABNORMALITY	REMARKS
				II	III	S_x II III	II III	S_x	—	S_x II	II	S_x III	III		
1.	1–6	♂	X	S or C_y	D	√*	√	√	√	0	0	0	0	II–III	
"	7	"	"	"	"	√	0	0	√	√	0	0	√	X–II	
"	8	"	"	C_y	"	36	0	0	32	28	0	0	19	"	small piece or much non-disjunction
"	9	"	"	"	"	11	6	1	10	1	15	8	1	X–III	small piece or much non-disjunction
"	10	♀	Y	"	"	14	10	24	11	0	0	0	0	II–III	
"	11	"	"	"	"	23	10	14	25	0	0	0	0	"	
"	12	"	"	"	"	17	15	12	16	0	0	0	0	"	
"	13	"	"	"	"	14	6	23	11	0	0	0	0	"	some crossing over between D and translocation
"	14	"	"	"	"	12	23	33	5	0	1	2	3	"	some crossing over between D and translocation
"	15	"	"	S	"	8	1	0	8	1	0	0	0	Y–II–III	double translocation; some non-disjunction and crossing over
"	16	"	"	"	"	8	0	0	14	17	0	0	19	Y–II	
"	17	"	"	C_y	"	11	1	1	9	15	1	1	8	"	some non-disjunction
"	18	"	"	"	"	9	1	2	5	4	0	0	7	"	" " "
"	19	"	"	"	"	9	0	0	9	0	7	2	0	Y–III	
"	20	"	"	"	"	15	3	8	15	9	16	10	1	"	much crossing over
2.	21	♂	"	S	III pl‡	22	17	10	18	0	0	0	0	II–III	
"	22	"	"	"	"	25	14	1	10	0	0	0	0	"	
"	23	"	"	"	"	17	26	8	6	0	0	0	0	"	
"	24	"	"	C_y	"	18	16	8	9	0	0	0	0	"	
"	25	"	"	S	"	15	11	11	10	0	0	0	0	"	confirmed in further tests
"	26	"	"	"	"	8	13	12	13	6	7	0	0	"	hyperdiploid classes viable
"	27	"	"	"	"	3	3	4	0	0	0	0	0	"	confirmed in further tests
"	28	"	"	"	"	14	11	15	14	0	0	0	0	"	"
"	29	"	"	"	"	9	17	11	11	0	0	0	0	"	"
"	30	"	"	"	"	8	13	6	14	0	0	0	0	"	"
"	31	"	"	"	"	22	14	24	13	0	0	0	0	"	"
"	32	"	"	C_y	"	20	16	27	18	0	0	0	0	"	"
"	33	"	"	"	"	10	10	16	20	0	0	0	0	"	"
"	34	"	"	S	"	14	0	0	15	0	15	20	0	Y–III	"

TABLE 2. (*Continued*)

Progeny counts in F_1 cultures involving translocations and other abnormalities.

EXPERIMENT NUMBER	CASE NUMBER	SEX OF F_1 TESTED	SEX CHROMOSOMES TESTED	MARKERS USED IN		NUMBERS OF FLIES RECEIVING RADIATED CHROMOSOMES AS INDICATED BY MARKERS (S_x = *radiated* X OR Y)								TYPE OF ABNORMALITY	REMARKS
				II	III	S_x II III	II III	S_x	—	S_x II	II	S_x III	III		
2	35	♂	Y	*S*	IIIpl	1	0	0	1	0	3	5	0	Y–III	confirmed in further tests
"	36	"	"	C_y	"	2	0	0	2	0	7	6	0	"	"
3.	37	"	X	"aps	theca†"‡	10	10	37	53	0	0	0	0	II–III	multiple-mutants have low viability in this experiment
"	38	"	"	"	"	2	8	22	17	0	0	0	0	"	
"	39	"	"	"	"	3	11	13	25	0	0	0	0	"	"
"	40	"	"	"	"	17	15	47	60	0	0	0	0	"	"
"	41	"	"	"	"	28	26	21	37	0	0	0	0	"	"
"	42	"	"	"	"	27	29	33	49	0	0	0	0	"	"
"	43	"	"	"	"	16	12	28	30	0	0	0	0	"	"
"	44	"	"	"	"	9	12	16	27	0	0	0	0	"	"
"	45	"	"	"	"	6	7	37	47	0	0	0	0	"	"
"	46	"	"	"	"	9	10	29	27	0	0	0	0	"	"
"	47	"	"	"	"	10	16	25	32	0	0	0	0	"	"
"	48	"	"	"	"	10	0	0	47	0	0	0	0	X-II-III	double translocation
"	49	"	"	"	"	9	1	1	34	14	1	0	18	X–II	some non-disjunction
"	50	"	"	"	"	1	0	0	7	13	0	0	17	"	
"	51	"	"	"	"	5	0	0	49	0	11	21	0	X–III	
"	52	"	"	"	"	6	0	1	17	0	23	12	0	"	
"	53	"	"	"	"	0	0	10	14	0	0	14	17	dominant lethal in II	
"	54	"	"	"	"	1	0	24	32	0	0	0	0	dominant lethal and II–III	or contamination; not counted as translocation
"	55	"	"	"	"	0	0	72	67	0	0	0	0		

* The symbol √ indicates that some flies of class indicated were present in each of the cultures in question, but number was not recorded.

† "aps theca" = $a_7\ p_x\ s_p\ t_h\ s_7\ e^s\ r_o\ c_a$.

‡ The markers "III pl," "aps" and "theca" were present in the non-radiated P_1 eggs; the markers C_y, *S*, and *D*, when used, were present in the radiated P_1 sperm.

effects of radiation on immature female germ cells and on spermatozoa indicates that the chromosome-abnormality frequency is increased much more in the spermatozoa as compared with the oögonia, than is the gene-mutation frequency. It is therefore possible that the high rate of translocations, as compared with gene mutations, observed in the experiments reported in the present paper, is a phenomenon especially pronounced in the spermatozoa.

Although the above consideration might partially account for the discrepancy in question, it is probably by no means sufficient to explain all of it, for undoubted displacements of chromosome sections have been found following irradiation of other cells than spermatozoa. It is therefore likely that there is a real difference between X-rays and the "natural" cause or causes of mutations, in regard to their relative effectiveness in altering the genes and in causing breakages and reattachments of parts of chromosomes. This conclusion is strengthened by the evidence, obtained by MULLER and MOTT-SMITH since the present paper was written, that natural radioactivity is not the cause of most mutations in untreated Drosophila.

SUMMARY

1 a. Methods are described for detecting the occurrence of translocations in Drosophila, based upon the principle that the effect of genic disproportion (excess or deficiency) involving even sections of chromosomes will often be sufficient to cause the inviability or abnormal appearance of one or both recombination classes of zygotes.

b. The results obtained by the use of these methods amply demonstrate the validity of the theoretical conceptions upon which they are based.

2 a. The frequency of translocations following heavy irradiation by X-rays has been found to be surprisingly high. In all, in the experiments herein described, 117 translocations, all or nearly all of them involving at least one breakage of one of the long autosomes and the attachment of a fragment of it to a non-homologous chromosome, have been found in a total of 883 fertile P_2-F_2 cultures derived from irradiated P_1 males.

b. The percentage of such breaks and reattachments of a given long autosome is thus at least 7 percent. Unit for unit of the chromosome, and dosage for dosage, this is just about half the rate of occurrence of detectable mutations of all kinds, as found in the X chromosome.

c. When allowance is made for the fact that translocations involving the attachment of the fiber-bearing instead of the fiberless fragment are not included in the above figure of 7 percent and that translocations too small to

EXPLANATION OF TABLE 2

This table can be explained by considering specifically experiment No. 1, cases 1–6. The sex of the F_1 fly tested was male, as indicated in column 3. The sex chromosome tested, as indicated in column 4, was the X, because the radiated father was mated to an attached X female; he therefore transmitted his X (rather than his Y) to his F_1 male offspring. The markers used in chromosomes II and III, as indicated in columns 5 and 6, were *S* (star) or C_y (curly), for chromosome II, and *D* (dichaete) for chromosome III. These genes are dominants. The F_1 male receives two of them, *S* (or C_y) and *D* from his radiated male parent. He receives the normal allelomorphs, *s* (or c_y) and *d*, from his normal, unradiated mother. In the F_1 males, sperm cells of four classes (so far as these markers are concerned) are formed by Mendelian segregation, namely, *SD*, *Sd*, *sD*, and *sd* (or C_y in place of *S*), and these contain, respectively, the radiated II and III chromosomes, the radiated II and unradiated III, the unradiated II and radiated III, and the unradiated II and unradiated III. When these F_1 males are mated to normal females, offspring (F_2) that recieve the radiated II and III chromosomes will appear *SD* (that is, star dichaete). Those that receive the radiated II but the normal III will appear *Sd* (star, not-dichaete), etc. Column 7 in the table is headed by the caption S_x II and III, and experiment 1, cases 1–6, are checked for these chromosomes; that is, there appeared some flies in the F_2 that were star and dichaete, and that therefore received II and III from their radiated grandparent. Being male, they also received a sex chromosome (S_x) from him. The next column is headed II and III, and below it is a check. This means that some flies appeared that received II and III from their radiated grandparent, but that, being female, they did not receive his radiated sex chromosome, as indicated by the absence of the symbol S_x in this column. The ninth column is headed "S_x" and again is checked below. This means that some flies appeared which received S_x, but neither II nor III from their radiated grandparent. The dash in the following (tenth) column, with a check below, indicates that some F_2 flies appeared that had not received S_x or the II or III from their radiated grandparent. In the eleventh column, headed S_x II, there is an O. This indicates that no flies appeared in the F_2 that received the sex and the II, but not the III, from the radiated grandparent. In the twelfth column, headed simply by II, there is also an O, showing that flies which failed to receive the radiated sex chromosome, but received the radiated II, and not the radiated III, likewise were missing. Columns 11 and 12, taken together, thus show that, regardless of the sex chromosome, flies with II but without III from their radiated parent could not live. In a similar manner, columns 13 and 14 indicate that flies that received only the III chromosome of their radiated grandparent, and not his second also, could not live. In other words, in experiment 1, cases 1–6, flies that received II without III, or III without II, could not live and did not make their appearance in the F_2. This fact indicates a translocation from II to III or from III to II, and is accordingly recorded as such in column 15, under the heading "Type of abnormality." In the remaining experiments, after case 7, (namely, experiments 1, 2 and 3, cases 8 to 55), the various viable classes of F_2 offspring were counted, instead of being simply "checked" for the appearance of a fairly large number of offspring belonging to these classes. The counts are indicated in columns 7 to 14.

result in readily detectable effects of the genic disproportion also are not included, whereas all kinds of chromosome abnormalities as well as "point mutations" involving lethal or other phaenotypic effects are included in the count of lethals in the X, it is seen that the frequency of occurrence of induced translocations is probably at least as high as, or higher than, that of induced gene mutations of a detectable kind.

d. This finding requires reconcilement with the apparent rarity of "natural" translocations, as compared with "natural" gene mutations.

The explanation may partly lie in an especial liability to chromatin displacements on the part of spermatozoa, which were the type of cells chosen for treatment. But it is also probable that the natural causes of mutations differ from X-rays in that they cause a much higher frequency of gene mutations, as compared with chromatin rearrangements, than do X-rays.

3. In the second experiment reported it was demonstrated that the translocations were not previously present in the stock, but arose in the cells of the treated generation.

4 a. A fragment broken off of either of the long autosomes (chromosomes II or III) can become attached to any of the other chromosomes present, namely, the other long autosome, the X, the Y, or the small fourth chromosome.

b. The relative frequencies of occurrence of the above various kinds of attachment, as found in the present experiments, formed the following ratios, respectively: (to II or III) 1: (to X) 0.45: (to Y) 0.22: (to IV) 0.15.

c. Among these differing frequencies, we may at least attach significance to the greater value of the first frequency (representing attachments to the other long autosome) as compared with each of the other frequencies (representing attachments to the smaller chromosomes).

d. The reason for this greater tendency for attachment to a long autosome is still undetermined, though possible explanations are suggested. Thus it may be that a breakage occuring in one chromosome favors the attachment to it of a fragment from another chromosome, and that breakage is more likely to occur in a longer chromosome. Breakage of a recipient chromosome is, however, not *necessary* for the occurrence of attachment.

5 a. Breakages of and translocations from the X chromosome have been found to occur in other work of the authors, although the technique of the present experiments largely prevented their detection here.

b. Breakages and translocations of part of the Y to other chromosomes seem, according to some tests herein reported, to be less frequent than those of the long autosomes (none having been found in a rather limited series of tests).

6 a. It has been found that a large proportion of the induced translocations produce lethal effects on zygotes which receive them in a homozygous condition. Those which are not lethal commonly produce sterility and other somatic manifestations, when homozygous.

b. Three possible reasons for such effects have been discussed—simultaneous mutation at a separated locus, possible effect on gene functioning of a

change in its gene associates, and mutation or loss of genes at or adjoining the points of breakage (or attachment). At present the last hypothesis seems the most probable.

c. These lethal and other deleterious effects of most translocations, when homozygous, do not rule out translocations as factors in evolutionary change any more than the lethal or deleterious character of the vast majority of detectable gene mutations rules out gene mutations as the main building blocks of evolution. It is not to be expected that the majority of any changes occurring at random willl have survival value.

TABLE 3

Results from individual P_1 males in portion of second experiment.

A DESIGNATION OF P_1 MALE	B MARKER CARRIED BY P_1 MALES	C NUMBER OF FERTILE P_2–P_3 CULTURES FROM "A", CARRYING MARKER INDICATED IN "B".	D NUMBER OF CULTURES IN "C" CARRYING TRANSLOCATIONS	E TYPE OF TRANSLOCATION	F CASE NUMBER OF TRANSLOCATION, AS REFERRED TO IN TABLE 2
1	*S*	5			
2	*S*	3	1	II–III	25
3	*S*	2			
4	*S*	12	1	II–III	26
5	*S*	7	1	II–III	27
8	*S*	2			
9	*S*	3			
10	*S*	6	1	II–III	28
11	*S*	5	2	II–III	31
				Y–III	34
12	*S*	3			
13	*S*	2			
15	*S*	1			
16	*S*	6	1	II–III	29
17	*S*	6	1	II–III	30
	C_y	3	1	II–III	32
18	C_y	3	1	II–III	33
	S	3			
19	*S*	2			
	C_y	6*			
20	*S*	2	1	Y–III	35
	C_y	2	1	Y–III	36†
Total		84	12	9II–III; 3Y–III	

* Dominant minute bristles, completely linked with C_y, appeared in 1 culture.

† Different translocation from number 35.

LITERATURE CITED

BLAKESLEE, A. F., 1927 Nubbin, a compound chromosomal type in Datura. Ann. N. Y. Acad. Sci. 30: 1–29.

BRIDGES, C. B., 1923 The translocation of a section of Chromosome II upon Chromosome III in Drosophila. (Abstract) Anat. Rec. **24**: 426.

HANSON, F. B., and HEYS, F., 1929a An analysis of the effects of the different rays of radium in producing lethal mutations in Drosophila. Amer. Nat. **63**: 201–213.

HANSON, F. B., and HEYS, F., 1929b Duration of the effects of X-rays on male germ cells in *Drosophila melanogaster*. Amer. Nat. **63**: 511–516.

HARRIS, B. B., 1929 The effects of aging of X-rayed males upon mutation frequency in Drosophila. J. Hered. **20**: 299–302.

METZ, C. W., 1914 Chromosome studies in the Diptera I. J. Exp. Zool. **17**: 45–49.

METZ, C. W., 1916 Chromosome studies in the Diptera III. Amer. Nat. **50**: 578–599.

MULLER, H. J., 1927a Artificial transmutation of the gene. Science **66**: 84–87.

MULLER, H. J., 1927b The effects of X-radiation genes and chromosomes. (Abstract) Anat. Rec. **37**: 174.

MULLER, H. J., 1928a The effects of X-radiation on genes and chromosomes. Science **67**: 82.

MULLER, H. J., 1928b The problem of genic modification. Proceedings of the 5th International Genetics Congress, Supplementband of the Z. indukt. Abstamm.- u. Vererblehre **1**: 234–260.

MULLER, H. J., 1928c The production of mutations by X-rays. Proc. Nat. Acad. Sci. Wash. **14**: 714–726.

MULLER, H. J., 1929 The gene as the basis of life. Proc. Internat. Congress Plant Sci. (1926) **1**: 897–921.

MULLER, H. J., and ALTENBURG, E. 1928 Chromosome translocations produced by X-rays in Drosophila. (Abstract) Anat. Rec. **41**: 100.

MULLER, H. J., and MOTT-SMITH, L. M., 1930 Evidence that natural radioactivity is inadequate to explain the frequency of "natural" mutations. Proc. Nat. Acad. Sci. Wash. **16**: 277–285.

MULLER, H. J., and PAINTER, T. S., 1929 The cytological expression of changes in gene alignment produced by X-rays in Drosophila. Amer. Nat. **63**: 193–200.

OLIVER, C. P., 1930 The effect of varying the duration of X-ray treatment upon the frequency of mutation. Science **71**: 44–46.

PAINTER, T. S., 1928 A comparison of the chromosomes of the rat and mouse with reference to the question of chromosome homology in mammals. Genetics **13**: 180–189.

PAINTER, T. S., and MULLER, H. J., 1929 The parallel cytology and genetics of induced translocations and deletions in Drosophila. J. Hered. **20**: 287–298.

SEREBROVSKY, A. S., DUBININ, N. P., AGOL, I. I., SLEPKOV, V. N., and ALTSHULER, V. E., 1928 Polucheniye mutatsii Roentgenovskimi luchami u Drosophila melanogaster. J. Exp. Biol. (Russ.) **4**: 161–180.

STERN, C., 1926 Eine neue Chromosomaberration von Drosophila melanogaster und ihre Bedeutung für die Theorie der linearen Anordnung der Gene. Biol. Zbl. **46**: 505–508.

STERN, C., 1927 Die genetische Analyse der Chromosomen. Naturwissenschaften **15**: 465–473.

STURTEVANT, A. H., 1921 A case of rearrangement of genes in Drosophila. Proc. Nat. Acad. Sci. Wash. **7**: 235–237.

STURTEVANT, A. H., 1925 The effects of unequal crossing over at the bar locus in Drosophila. Genetics **10**: 117–147.

STURTEVANT, A. H., and PLUNKETT, C. R., 1926 Sequence of corresponding third-chromosome genes in *Drosophila melanogaster* and *D. simulaus*. Biol. Bull. **50**: 56–60.

WEINSTEIN, A., 1928 The production of mutations and rearrangements of genes by X-rays. Science **67**: 376–377.