THE REGIONALLY DIFFERENTIAL EFFECT OF X RAYS ON CROSSING OVER IN AUTOSOMES OF DROSOPHILA[1]

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

Received March 28, 1925

TABLE OF CONTENTS

THE PROBLEM

It has been stated by MAVOR (1924) that "We see in the effects which X rays produce on crossing over in the first and second chromosomes of Drosophila a remarkable instance of opposite effects. . . . ." This statement was based on extensive and critical data, which showed clearly that there was a significant decrease in crossing-over frequency between white and miniature, in the first chromosome, but a significant increase between black, purple and curved, in the second chromosome, after application of similar doses of X rays to both. MAVOR (1924) further noted that in the case of the black-purple and purple-curved regions, "the greatest effect for the same dose is produced in the black-to-purple region,"—and, as calculations to be reported in the present paper now prove, the differences between the effects observed by MAVOR on these two regions of the second chromosome are statistically significant. This observation, however, raises the question whether crossing over in still other regions of the same chromosome (II) might not show even greater differences in the effects of X rays than those shown between the black-purple and purple-curved regions. If so, a comparison could scarcely be drawn between the

[1] Contribution No. 193 from the Department of Zoölogy, of the UNIVERSITY OF TEXAS.

effects of X rays on different chromosomes as a whole, inasmuch as the crossing-over reactions would be highly specific for individual chromatin regions considerably smaller than those blocks of chromatin comprising the morphological chromosomes.

An investigation concerned with the above problem,—i.e., designed to reveal possible intrachromosomal differential effects of the X rays on crossing over,—had been begun by the present writer before the suggestive results on black-purple-curved, quoted above, came to hand, for certain already-existing data had made the finding of such differences not unlikely. The reasons for suspecting such effects may be listed under two heads, cytological and genetic.

In regard to the cytological evidence, it is to be noted that the central regions of the long V-shaped autosomes (chromosomes II and III) are morphologically different from the distal arms of the same chromosomes in at least four respects: (1) The spindle fibre is attached in the central regions, and not in the distal; (2) The bend of the chromosome regularly occurs in the central regions; (3) The chromatin appears to be less deeply staining there; (4) It is narrower in this vicinity.

The purely genetic grounds for expecting regionally differential chromosome reactions are as follows: (1) PLOUGH (1917, 1921) had shown that extremes of temperature affected crossing over in the central regions of both the long autosomes, but not in any of the distal regions which he studied; the latter behaved like the greater portion of the rod-like X chromosome in this respect. (2) BRIDGES (BRIDGES 1915; BRIDGES and MORGAN 1919, 1923) had made similar findings with regard to the effect of age on crossing over. (3) BRIDGES and the present author had noted that the central regions of the autosomes have a higher coincidence of crossing over than the distal regions,—which in this feature also resemble the X chromosome more closely. (4) The standard maps, based on the collective Drosophila work (given in BRIDGES and MORGAN 1919 and 1923) show a tendency to a concentration of mutant genes in the central regions of both chromosomes. (5) The same effect appears decisively in the maps of lethal genes in chromosome II found in the present writer's mutation studies (MULLER 1920). This apparent crowding effect in the middle regions might be due either to mutations occurring more frequently there than in the distal regions, or to a relatively lower frequency of crossing over in the middle regions. Of these two possible explanations the latter is by far the more probable.

All told, the above facts furnish a considerable body of evidence which demonstrates that the crossing-over reactions of the chromosomes

throughout their length cannot be studied through data based on limited regions of them. And it is evident, specifically, that the central regions of the long autosomes have crossing-over properties which distinguish them especially from other portions of the chromatin. Now, it so happens that the loci of black, purple and curved, used by MAVOR, all lie near the middle of the second chromosome. Results based on these loci, therefore, must be regarded as particularly unrepresentative of the reactions of the rest of this chromosome, and before we can judge of the crossing-over reactions to X rays of this chromosome, in general, in comparison with reactions of other chromosomes, it is necessary to study distal as well as central regions.

Experiments directed expressly towards a study of the differential effect of X rays on the different regions within the large V-shaped autosomes were carried out with both the second and third chromosomes. It was necessary, in this work, to follow the distribution of a considerable number of loci, scattered throughout the major portion of the lengths of these chromosomes in such a way as to include and differentiate between the middle and other regions, and extending distally to one end at least. It was preferable too that all the loci studied in a given chromosome should be followed simultaneously, so that differences in crossing-over frequency due to uncontrolled factors that might vary from experiment to experiment would be avoided. Such an arrangement would, in addition, permit a more accurate study of coincidences of crossing over, and of the effect of X rays upon the coincidences involving various regions.

It was also of interest to determine whether or not chromosomes otherwise identical might respond differently to X rays according to whether other factors affecting crossing over,—such as, for instance, specific genes hindering crossing over,—were different. Hence, a variation was introduced into one of the experiments by using a chromosome containing one of these genes.

Finally, it was thought desirable to vary to some extent the X-ray dosage employed, so that possible variations in the effect on different regions due to this cause might be observed.

TECHNIQUE

A multiple-recessive stock was used for the second-chromosome study, containing the six mutant genes dumpy (T^d), black (b), purple (p_r), curved (c), plexus (p_x) and speck (s_p). This stock, which had been made up chiefly by BRIDGES, and to which T^d had been added by the author, may be referred to here as "II-ple." The plexus character was not followed

in the counts. The positions of these loci in the standard map (BRIDGES 1921, unpublished) are shown in figure 6. It will be seen that the first gene is 8 units from the "left-hand" end and the other genes scattered over the remainder of the chromosome all the way to the extreme "right-hand" end. In the mid-region, which requires more minute study, the genes are more closely spaced.

For the third chromosome a multiple-recessive stock designated as "III-ple" was used. This stock, made up chiefly by BRIDGES, contains roughoid (r_u), hairy (h), scarlet (s_t), pink (p), spineless (s_s) and ebony (e). The positions of these genes on the standard map (BRIDGES and MORGAN 1923) are shown in figures 4 and 5; the first gene is at the extreme left-hand end and the others scattered, up to about 30 units from the right-hand end. Here, too, there is closer spacing in the middle and a rather wide even spacing elsewhere. In the case of both stocks the loci closely on either side of the mid-point (b and p_r, and s_t and p, respectively) are so located as to include a larger section of chromatin to the left than to the right of the center, and there are other loci (c and s_s, respectively) bounding a region (p_r-c and p-s_s) somewhat farther to the right of the middle, yet appreciably closer to the latter than is the region (T^d-b and h-s_t, respectively) to the left of the most central region. Farthest of all from the center, in each case, are the remaining regions, c-s_p in II and s_s-e and r_u-h in III, the first two named being to the right, the last to the left of the center. Hence, in each case there are several regions for study, increasingly distant from the center, and at distances from it that are comparable in the two stocks.

In the first experiments, in order further to make the data comparable, flies of as nearly identical composition as practicable were secured for the studies on the two chromosomes. This was effected by crossing flies from the II-ple stock to III-ple flies (heterozygous for the second-chromosome gene curly, C_v, which will be referred to later) and using the female offspring, heterozygous for both sets of genes, for either study, in the one case by backcrossing them to II-ple males, and in the other case by backcrossing to III-ple males. Identical food and environic conditions were used, of course, in all cases. The controls and the individuals to be X-rayed, in the case of the flies of both second- and third-chromosome experiments, were gotten by subdividing the latter groups of flies by random selection, and the control and X-ray lots so obtained were in each case subjected to identical conditions, apart from the raying. As flies from the same bottle, all of similar age, were employed throughout, and (after separation into curly and non-curly) divided at random between

the treated and control lots, it will be seen that the flies in any of the treated lots were genetically and developmentally as much like those in the corresponding control as they were like each other.

Both control and X-ray lots of flies were kept virgin until the time of raying, when all were between one and two weeks old. After the raying they were mated and kept in a first set of bottles for six days, and then transferred to other bottles. The offspring from the first bottles were discarded, inasmuch as MAVOR has shown that the effect of raying on crossing over does not commence immediately, and it was desired to secure results which would be homogeneous. Each female was placed, with one male, in a separate bottle, when the cultures that were to have counts made upon them were made up, and separate records were kept of the results from each pair. The offspring counted were derived from the eggs laid from the sixth to the twelfth day after raying.

On the female flies referred to above, derived from the cross of II-ple by III-ple, heterozygous for curly, three sets of experiments were conducted. In experiment "a," one lot, consisting of 14 curly females (not counting those that proved sterile) were backcrossed to III-ple males, for a study of crossing over in the third chromosome. Seven of these were rayed and seven served as controls. (The non-curly flies were not used in this case, because the gene for black, present in the latter heterozygously, might have tended to confuse the classification of the offspring in regard to ebony). In experiment "b" another lot, consisting of 18 (fertile) non-curly females were backcrossed to II-ple, for a study of crossing over in the second chromosome; 10 of these were rayed. In experiment "c" a third lot, consisting of 15 (fertile) curly females, were backcrossed to II-ple, and of these 5 were rayed. In this third lot an inhibition of crossing over was expected to occur, owing to the presence of curly, which has been found by WARD (1923) greatly to reduce the frequency of crossing over in the second chromosome,—without, however, affecting it in the third chromosome.

A much weaker dose of X rays was applied than that used by MAVOR, in order not to affect fertility adversely and also to bring to light any difference in effect from that observed by him, which might be produced by a lesser dose of the rays. PLOUGH (1924) has recently suggested that smaller doses may have an effect on crossing over opposite to that produced by larger doses, and that a smaller dose, in the case of a chromosome more sensitive to the rays (as he suggests that chromosome II may be) would then produce a similar effect (decrease of crossing over) to that observed in the case of a less sensitive chromosome (the X chromosome)

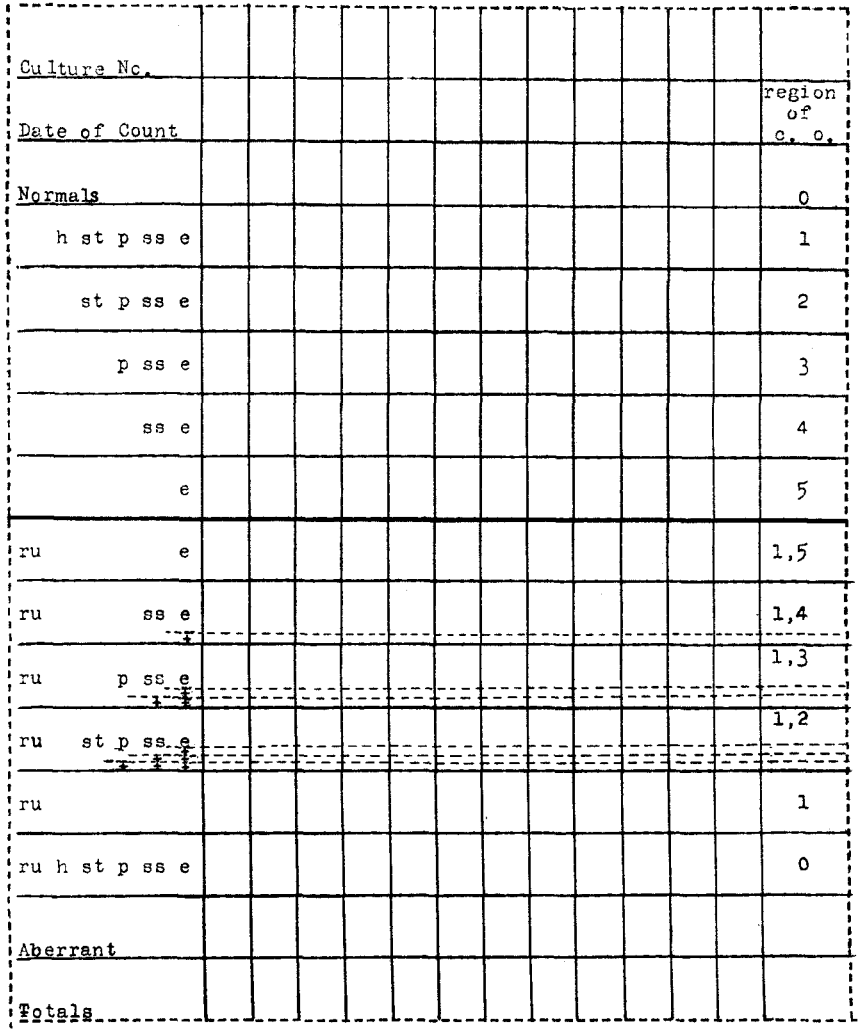
when a large dose is applied. If such a relation held, then, it might be revealed when a small dose of X rays was applied.

The dose used in the experiments here referred to may be designated as 26.8 H (Holzknecht units). One H, or Holzknecht unit, may be described as the amount of radiant energy received from unfiltered rays resulting from a current of 3 milliamperes, at 30,000 volts, when applied for 1 minute at a distance of 20.3 cm (8 inches). The radiant energy received, in number of H, may then be calculated by the formula: $\frac{\text{volts} \times \text{milliamperes} \times \text{minutes}}{(\text{centimeters})^2} \times .00458$. (One H is then equal to .437 of MAVOR's "D," when 50,000 volts are used.) In the present work unfiltered rays from a broad-focus Coolidge tube with tungsten target were employed throughout, and the voltage was kept at 50,000. In experiments a, b and c, the flies of which were treated simultaneously, the number of milliamperes averaged 4, the time 7.5 minutes, and the distance 16 cm. The total energy absorbed by the tissue in question, rather than these individual items, has been shown usually to be the more significant factor in determining the degree of various biological effects of X rays, particularly if the spectrum is constant, and MAVOR's work has verified the truth of this rule with regard to the effect on crossing over, specifically. H gives, of course, the amount received rather than absorbed, but since, with a given spectrum and a given tissue, the proportion of the received rays that are absorbed is constant, the dosage under circumstances like those in the present experiment may be expressed in H. The dose of 26.8 H used here was little more than half of the minimum dose, 48 H (21 "D"), used by MAVOR in his crossing-over work, and slightly less than a third of the dose designated as optimal by him, namely 80 to 86.8 H (35 to 38 "D").

It will be seen later that when the results of the above experiments were tabulated, some apparent differences between the results on both second and third chromosomes and the comparable portion of MAVOR's work on the second chromosome appeared. In order to obtain evidence as to whether these differences were really due to the difference in X-ray dosage, and also in order to secure, if possible, more marked effects on the third chromosome, with a dose similar to that which MAVOR had used on the second chromosome, the part of the experiment relating to chromosome III was then repeated (experiment "d"), with the larger dose of 54.4 H. This dose is somewhat larger than MAVOR's minimum dose and twice as large as the dose previously used by the present writer. The cross was made and the entire experiment was conducted as before, with the

following exceptions: (1) A different stock of II-ple was used, not containing curly; consequently, non-curly female offspring, heterozygous for

A



FIGURES 1, 2 and 3.—Tabulation forms for complex linkage counts (backcross of $\frac{r_u\, h\, s_t\, p\, s_s\, e}{+}$). Directions for fitting forms together are given in the text.

II-ple and III-ple, were bred, being backcrossed to the III-ple males. Fourteen females were treated, and eight kept as controls. (2) The females

were three weeks old when bred. (3) The offspring hatched resulted from eggs laid during a period of four to twelve days after raying. (4) Two females were bred in each bottle. Owing to the first two differences the crossing-over frequencies in the controls of this experiment were significantly different from those in the controls of the preceding experiment.

In the account that follows, the results of the experiments will be reported in the order a, d, b, c, as this sequence is better adapted for a comparison of significant features.

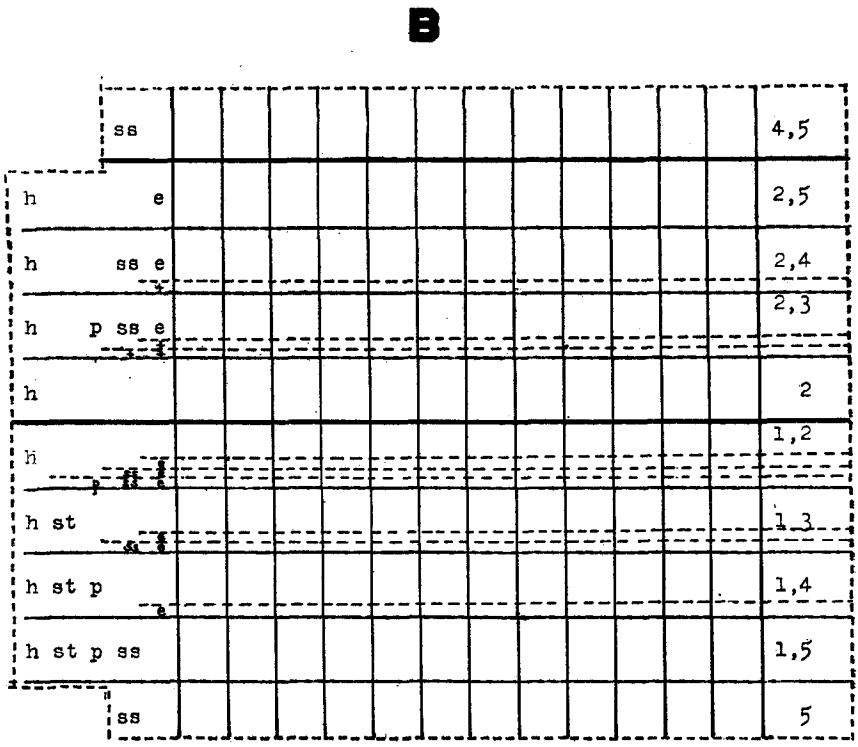


Fig. 2

In recording counts like those made here, which involved either five or six, usually six, pairs of characters, and 32 or 64 possible combinations of characters, the tabulation is ordinarily very cumbersome and time-consuming,—a fact which seriously limits the numbers which it is feasible to count. An effort was therefore made to devise a more convenient system of tabulation, and a scheme was arrived at which proved so useful that it may be given here (figures 1 to 3) for the benefit of any workers who have similarly complicated linkage counts. The scheme as here

presented is for the recording of 6-locus third-chromosome counts, from females having III-ple in one third chromosome and all normal genes in the other, but it can of course be adapted, by changing and rearranging letters, to any other 6-locus count, and a similar scheme may be used for any number of loci.

The tabulating form is made by cutting out the three pieces along their outer dotted lines, and folding along the heavy lines, as follows. Each of the two smaller pieces (B and C) is first folded back along its mid-line

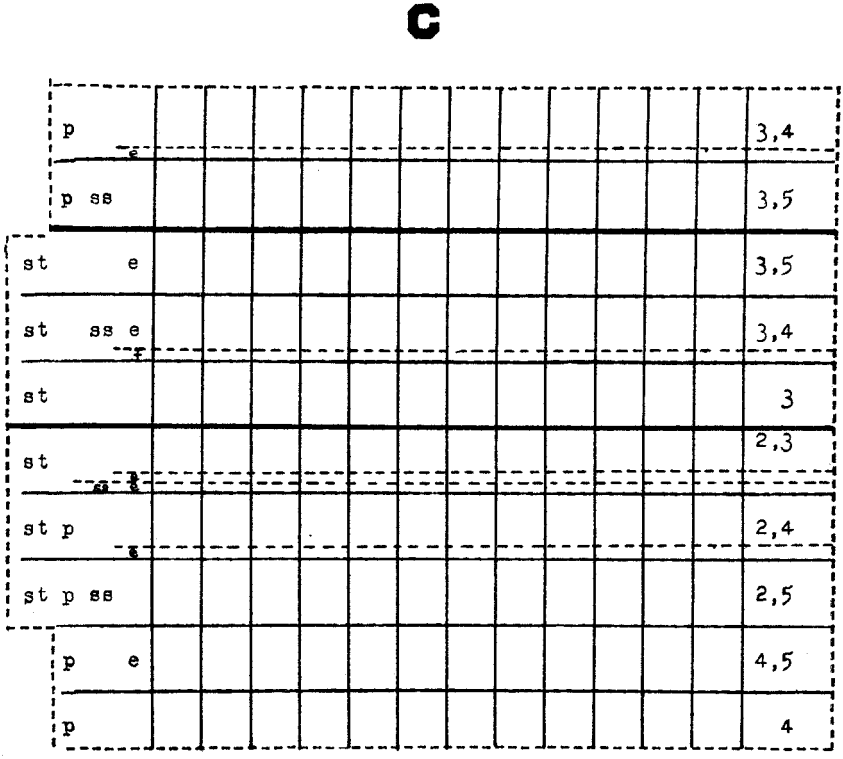


Fig. 3

and its two apposed halves are pasted, or held together with labels. Each (doubled) piece is then held with that side upward on which the remaining heavy line appears, and so placed that the letters seen are upright. The piece is then folded over towards the manipulator along this remaining heavy line, by leaving the lower, larger section of the piece in place and inverting the smaller, upper section over it, so that the back of the latter appears in view (with its letters now in upright position). This fold is not pasted together but left as a movable hinge. Piece B is

then attached to A along its movable hinge by means of labels, in such a position that the hinge lies along the central line of A, and identical letters of the two pieces lie in the same vertical line. Similarly, piece C is fitted into piece B, within its movable fold, so that its hinge lies along the same line as that of B and its identical letters are in line with those of A and B. When B and C are both in their folded, or more usual position, C then lies attached within the fold of B.

The advantage of this form lies in the fact that the combinations most frequently encountered—non-crossovers and single crossovers—are compactly and systematically arranged in open view, for ready recording of results, and in addition the other classes are not scattered along so that a search is necessary for finding them, but are immediately exposed by the left hand when the finger is inserted in the space between the letters representing the first two genes between which crossing over has occurred. Thus, if a double crossover commencing r_u h non-s_t is found (this involves a crossing over first between h and s_t) the finger is inserted upon r_u h, so as to remove s_t, by folding upwards the sheet containing this symbol. This brings to view, at the point of the finger, the space for recording such double crossovers. On the other hand, if the double crossover had been non-r_u non-h s_t, the finger, isolating s_t from r_u h, and following it upwards, points to the record of all double crossovers commencing in s_t. If one thinks of the characters in the order in which their corresponding genes lie in the chromosome, the finding of each given combination on the table by the above method soon becomes practically automatic. The convenience of the form of tabulation here described, and the ease with which a given class is found by means of it, is, however, more readily appreciated after a little manipulation of the form than by long explanations. It will likewise be found that this form saves much time when the results are treated for analysis, as the employment of the third dimension makes possible a more systematic juxtaposition of corresponding classes, and a readier summation or comparison of them than can be secured in the ordinary table. Forms of this type, once worked out, may be mimeographed, and are then available for extensive counts.

In taking the record, it saves time first to pick out the flies of the classes expected to be most numerous (the non-crossovers), to count and record these and get them out of the way, then to proceed to the next most numerous, and so on down, for this results in looking over the same fly the smallest number of times. Another way is simply to keep tally, by classifying each fly in succession as it comes and recording this with a mark in the appropriate square of the table. In that case the time-saving

feature of the present as compared with other forms of tabulation becomes most marked, but tallying is not advisable unless the proportion of classes to number of flies counted is very large.

The writer is deeply indebted to Doctor DALTON RICHARDSON, Roentgenologist of Austin, Texas, for the generous spirit of scientific coöperation in which the latter carried out the actual treatment of the flies, with his own apparatus.

In addition, the writer wishes to acknowledge the efficient and conscientious aid given him by his students, Mr. G. LANGNER, Mr. H. LEFKOWITZ, Miss A. RUYSENAARS and Miss F. SETTLES, in the making of the counts in the present experiments involving the lighter dose.

DATA ON REACTION OF THIRD CHROMOSOME TO LIGHTER DOSE

In table 1, data from the experiment on the third chromosome, involving the series treated with a dose of 26.8 H (11.7 D), and its control, are presented in condensed and analytic form, the percent of crossing over, or "distance," between each two neighboring genes being given, and also

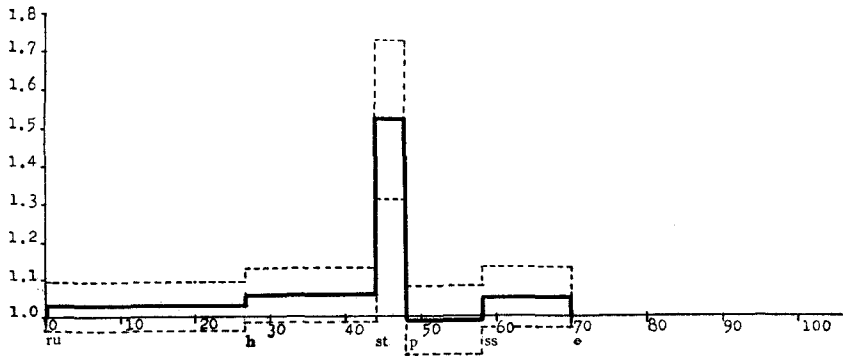


FIGURE 4.—Effect of lighter dose on chromosome III. Heavy line represents quotient of treated divided by control crossover value, height of line (ordinates) giving size of quotient for region of chromosome shown on base line (abscissae). Upper and lower dotted lines give values of quotient plus and minus its probable error. Points on base line are plotted according to the standard map values.

the total percent of crossing over, or "map length," and the total number of flies counted. After each of the percents is given its "probable error" (to which it would be subject if random samples of the given size were taken out of an indefinitely large lot of individuals having the given percent as its true percent). The values for the treated series are given on the first line, those for the controls on the second line. The differences between percents for treated and control series, with their "probable errors" (taken on a basis corresponding to that above) are given on the

GENETICS 10: S 1925

TABLE 1

Effect of lighter dose on crossing-over frequency in chromosome III.

ORIGIN OF DATA	PERCENT OF CROSSINGS OVER IN:						NUMBER COUNTED
	Region 1 (r_u–h)	Region 2 (h–s_t)	Region 3 (s_t–p)	Region 4 (p–s_s)	Region 5 (s_s–e)	Total distance (r_u–e)	
Treated series, "T"	25.1±1.19	22.1±1.14	7.3±.71	12.4±.90	17.2±1.03	84.1±1.86	605
Control series, "C"	24.3±.97	20.8±.90	4.8±.48	12.5±.74	16.4±.82	78.8±1.73	917
Difference, T−C	+1.8±1.54	+1.3±1.45	+2.5±.87	−0.1±1.17	+0.8±1.32	+5.3±2.5	
Difference ÷ probable error	1.2	0.9	2.9	0.09	0.61	2.1	
Quotient, T÷C	1.03±.064	1.06±.071	1.52±.212	.99±.093	1.05±.082	1.07±.033	

third line, and the number representing each difference divided by its own probable error is recorded on the fourth line. On the fifth line the ratios, or quotients, formed by dividing the percent for the treated by that for the control series, are given, with their probable errors (also on a corresponding basis); a graphic representation of the quotients and their probable errors is shown in figure 4.

Examination of the table shows at once that the only region in which a difference at all significant occurred between treated and control series was s_t-p, or region 3, the section which is believed to include the middle point of the chromosome (at which the spindle fibre becomes attached). The differences in all the other regions were scarcely, if any, greater than their own probable errors, but in this middle region the difference was of a size (2.9 times its probable error) such that it would have occurred only once in twenty times had the treated and control really had the same crossing-over value. The difference was, moreover, in the same direction as that found by MAVOR for the central region of the other long autosome, —that is, an excess of crossing over in the treated series. It is, therefore, probable (with a probability of 19 to 1, according to the usual method of reckoning) that a dose of X rays of only about 27 H increases the frequency of crossing over in the center of the third chromosome. There is no evidence, however, that this dose of X rays affects crossing over elsewhere in the third chromosome, either by increasing or decreasing it, and it is noteworthy that this statement applies to p-s_s, the region next nearest the center, at least as strongly as to the regions further removed, whereas in MAVOR's work on the second chromosome, in which a larger dose was used, the region, p_r-c, corresponding to p-s_s, was affected decidedly, and in the same sense as the most central region.

Although the ratios of treated to controls are, of course, subject to considerable error, they are a measure of the intensity of the effect produced by the treatment,—so far as this may be revealed by the data at all, —and, as will be seen later, it will be desirable to compare these ratios statistically both with each other and with the ratios obtained in the other experiments.

The total map lengths of the chromosome sections studied show an excess of 5.3 units in the treated series. Although such a difference (2.1 times its probable error) should occur, on the average, once in every six samples of the given sizes even if the true map lengths were the same, nevertheless, it may be taken as slight evidence in favor of the total map length being increased by treatment. The increase found, however, gave the treated series a length of only 1.07 ± 0.3 times that of the controls,

GENETICS 10: S 1925

TABLE 2

Effect of lighter dose on coincidence in chromosome III.

ORIGIN OF DATA	REGIONS INVOLVED:					
	1 and 2	2 and 3	3 and 4	4 and 5	2 and 4	1 and 5
Treated series, "T"	0.57± .077	1.1± .205	2.4± .358	0.31± .104	1.2± .174	1.09± .133
Control series, "C"	0.60± .068	1.4± .242	1.1± .280	0.37± .085	0.8± .115	1.26± .10
Difference, T−C	−0.03± .102	−0.3± .32	+1.3± .45	−0.06± .135	+0.4± .21	−0.17± .166
Difference ÷ probable error	0.29	0.94	2.9	0.44	1.9	1.02

showing that the chromosome as a whole was, at any rate, not much affected.

The percents of crossing over, considered in each region, separately, are not the only values about which it is desirable to obtain information. The data also contain information regarding the amount of coincidence of crossing over, that is, the frequency of double crossovers, as compared with the proportion of double crossovers which would have occurred if crossings over in the two regions considered had been independent of one another (see MULLER 1916, WEINSTEIN 1918). The values of this "coincidence," for each two contiguous regions, and some others, are given in table 2. In the case of both treated and controls the coincidences between adjacent regions (involving distances of 0 to 30 or 40 for the double crossovers involved) are significantly below 1.0 (the expectation if crossings over in the two regions were independent) in the outer parts of the chromosome, but within the range of error of 1.0 in most cases involving even short distances in the middle of the chromosome. This result is in accord with those of previous experiments on the (non-X-rayed) third as well as second chromosomes. One coincidence, however, that for 3,4,—the middle and an adjacent region,—in the treated series seems significantly higher than 1.0. Much stress cannot be laid upon this fact, as the unavoidable rare errors which may be made in classification necessarily affect the accuracy of the small double-crossover classes far more than that of the other classes. The difference between the 3,4 coincidence values of treated and control is 2.9 times its probable error. As this occurs in the very region in which crossing over has been increased, it may represent a real difference. On the whole, however, the coincidences for adjacent regions are strikingly alike in treated and control series.

If the coincidences for adjacent regions approach 1.0, it might be expected that those for more distant regions would be still more likely to show this ratio connoting independence of crossings over. The coincidences of distant regions might, therefore, have less chance to show an effect from the treatment than those of adjacent regions. Those for 2,4,—the regions on either side of the center,—were calculated, as being most likely to show an effect from treatment, but, as the table shows, they do not depart significantly from 1.0 or from each other. Coincidences for 1,5 were also calculated,—as representing the most widely separated regions,—but here too they are within range of 1.0 and of each other.

In calculating these coincidences for regions not adjacent to each other, only that part of the data was allowed to enter into any of the elements of the computation, which comprised individuals not having a crossing over

TABLE 3

Effect of heavier dose on crossing-over frequency in chromosome III.

ORIGIN OF DATA	PERCENT OF CROSSINGS OVER IN:						NUMBER COUNTED
	Region 1 (r_u–h)	Region 2 (h–s_t)	Region 3 (s_t–p)	Region 4 (p–s_s)	Region 5 (s_s–e)	Total distance (r_u–e)	
Treated series, "T"	20.8±.67	20.0±.66	6.2±.40	10.7±.52	10.8±.52	68.5±1.19	1638
Control series, "C"	23.3±1.0	17.8±.89	3.1±.40	5.9±.55	11.4±.74	61.5±1.51	847
Difference, T−C	−2.5±1.2	+2.2±1.11	+3.1±.56	+4.8±.76	−0.6±.90	+7.0±1.92	
Difference ÷ probable error	2.08	2.0	5.5	6.3	0.7	3.65	
Quotient, T÷C	0.89±.048	1.12±.067	2.0±.289	1.8±.189	0.95±.076	1.11±.033	

in an intermediate region. The resultant coincidence coefficient, which may be called a "partial coincidence," shows more correctly the possible influences upon one another of crossings over in the two regions considered, than the ordinary coincidence coefficient, which entails the disturbing influence of intermediate crossings over, would do. The effect of the intermediate crossing over, being to "inhibit" crossing over both to left and right of it, causes a reduction in the number of crossings over that happen simultaneously in those two regions, and thus may cause an apparent negative correlation (reduction of coincidence below 1.0) between these distant crossings over, even though they may be having no direct

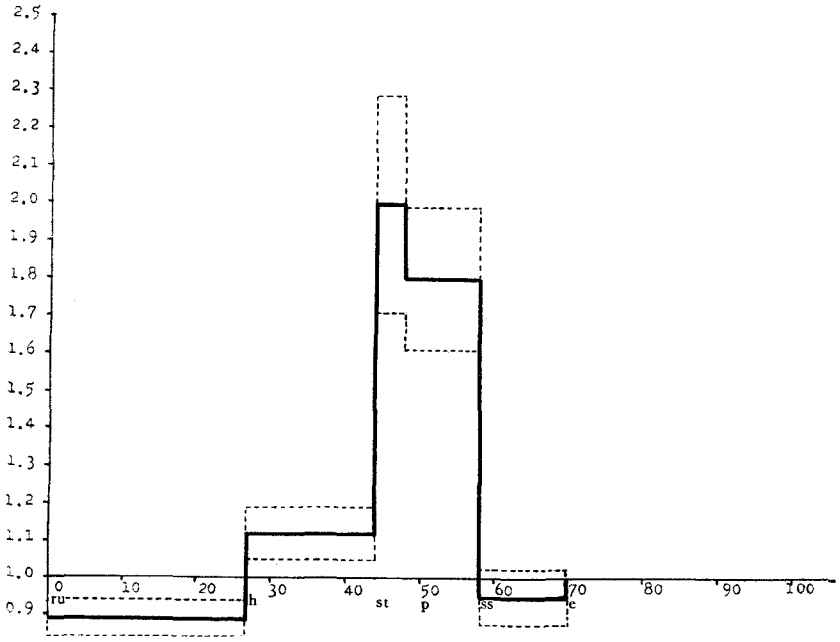


FIGURE 5.—Effect of heavier dose on chromosome III. Details as in figure 4.

influence on each other. The "partial-coincidence" coefficient, avoiding this source of error, has in our own experiments shown a coincidence within the range of error of 1.0 for the more distant regions in at least fourteen out of sixteen cases, whereas the ordinary coincidence would in most of these cases have been below 1.0.

DATA ON REACTION OF THIRD CHROMOSOME TO HEAVIER DOSE

The results obtained for crossing-over frequency in the third chromosome when the dose of X rays was doubled, and those of the control series bred simultaneously, are shown in table 3, in which the data are presented

in the same manner as in table 1, already described. Figure 5 is a graphic representation of the quotients of table 3, and their probable errors.

It will be seen that in this experiment, both the most nearly central region, s_t-p, and that next nearest to the center, p-s_s, showed a highly significant excess of crossovers in the treated as compared with the control counts. The chance that such differences should have been found in random samples of the same material is considerably less than 1 in 5000, in each case. On the other hand, the differences in the other regions, which, taken at their face values, suggest a slight decrease of crossing-over frequency in the more distal regions of the chromosome, have no distinct statistical significance.

The result, then, seems to prove that the effect of X rays in increasing crossing over, is, with larger doses, not confined to the very center of the chromosome. Hence, the effect could not be due to a mere increase in the number of breaks at a given point—such as the point of attachment of the spindle fibre. This result for the third chromosome is in accordance with the results obtained by MAVOR, with similar doses of X rays, for the second chromosome, inasmuch as he found both the central region (b-p_r) and one adjacent to the center (p_r-c) to be affected; in his work, however, as no other regions were studied, the question of a possible localization of the effect was not raised. In the present experiment there is also some slight evidence of an increase of crossing over in the region (h-s_t) adjacent to the center on the left, but the increase indicated is small, in accordance with the fact that this region averages considerably farther from the center than does the adjacent region on the right (p-s_s). The total increase indicated, for all the central regions, is 10.1 units with this dose, as compared with 3.7 units in the case of the dose half as large.

The total map length in the present experiment is 7 units greater in the treated than in the control series, a difference having distinct statistical significance (chance in random sampling 1 in 250), but somewhat smaller than the difference in the more central regions taken by themselves, owing to the slight decreases found in the distal regions. It may be noted here also that, owing to the fact that most of the coincidences remained approximately 1.0 in the treated series, this increase in map length did not carry with it any significant change in the percent of actual separations between the two most distant points considered, r_u and e, even when this relatively strong dose of X rays was employed; for the percent was 44.5 ± 1.2 in the controls and 43.9 ± 0.8 in the treated counts,—actually a (non-significant) decrease of 0.6 percent $\pm$ 1.42 percent in treated as compared with controls.

TABLE 4

Effect of heavier dose on coincidence in chromosome III.

	REGIONS INVOLVED				
	1 and 2	2 and 3	3 and 4	4 and 5	1 and 3
Treated series, "T"	0.37 ± .046	1.1 ± .126	1.0 ± .192	0.42 ± .095	0.88 ± .122
Control series, "C"	0.28 ± .056	0.22 ± .143	2.0 ± .712	0.18 ± .12	0.75 ± .202
Difference, T − C	+0.09 ± .073	+0.88 ± .19	−1.0 ± .74	+0.24 ± .153	+0.13 ± .24
Difference ÷ probable error	1.2	4.6	1.35	1.6	0.56

TABLE 4 (continued)

	REGIONS INVOLVED				
	2 and 4	3 and 5	1 and 4	2 and 5	1 and 5
Treated series, "T"	1.05 ± .10	0.86 ± .177	1.03 ± .10	1.02 ± .101	1.07 ± .111
Control series, "C"	0.70 ± .176	0.45 ± .174	1.44 ± .186	1.11 ± .141	0.625 ± .109
Difference, T − C	+0.35 ± .203	+0.41 ± .25	−0.41 ± .212	−0.09 ± .174	+0.445 ± .156
Difference ÷ probable error	1.72	1.61	1.93	0.52	2.85

The coincidences, which were all calculated, are shown in table 4, together with their probable errors, and the differences between treated and control coincidences, with their errors, and the number of times these differences are of their errors. In the case of non-adjacent regions the partial coincidences were obtained as before, instead of the gross coincidences, by elimination from the data of all crossovers occurring in regions lying between those considered.

It will be seen that the values obtained here are, on the whole, very similar to those of the preceding experiment, being low, distinctly below 1.0, for nearby regions in the distal parts of the chromosome, but within range of 1.0 in the great majority of the other cases. In all but two of the 10 pairs of cases, moreover, the values are substantially the same in treated and control series, the difference being not more than twice its probable error. One of these pairs of aberrant cases is coincidence 2,3, involving the middle and an adjacent region. This suggests, as did the aberrant difference for coincidence 3,4 in the first experiment, that in the central region the coincidence is being increased by X rays. Neither experiment really corroborates the other in regard to this question, however, since the conspicuous difference in each experiment is balanced by an equality of the exactly corresponding coincidences in the other experiment. Comparison of the controls and treated in the two experiments suggests that the difference between control and treated values of coincidence 2,3 in the present experiment may really be due to an abnormally low value for this coincidence in the present control, as the treated value of the present experiment is about the same as both treated and control values in the other experiment. The same consideration holds with regard to coincidences 1,5, in which the treated value in the present experiment conforms to the 1.0 value to be expected for such distant regions, and to the value obtained in both treated and control in the other series. After all, however, the difference between treated and control coincidences 1,5 in the present experiment does not represent a very rare chance for samples from the same material, being less than 3 times its probable error. And, as explained previously, coincidence values are likely to be subject to other errors than those of random sampling in greater degree than are crossing-over frequency values.

On the whole, then, the similarity of the coincidence values obtained in treated and controls, and of the values obtained in the two experiments, is the most striking feature of the coincidence results. The study does not include, however, regions near the center which are small enough and close enough together to have coincidence values which are normally

TABLE 5

Effect of lighter dose on crossing-over frequency in chromosome II.

ORIGIN OF DATA	PERCENT OF CROSSINGS OVER IN:					NUMBER COUNTED
	Region 1 (T^d–b)	Region 2 (b–p_r)	Region 3 (p_r–c)	Region 4 (c–s_p)	Total distance (T^d–s_p)	
Treated series, "T"	23.2±.64	8.73±.44	19.4±.61	26.8±.67	78.13±1.37	1969
Control series, "C"	21.6±.72	6.26±.42	17.9±.67	26.0±.75	71.76±1.39	1502
Difference, T−C	+1.6±.94	+2.47±.62	+1.5±.91	+0.8±1.0	+6.37±1.95	
Difference÷probable error	1.7	4.0	1.65	0.8	3.27	
Quotient, T÷C	1.07±.046	1.4±.12	1.08±.053	1.03±.04	1.09±.028	

distinctly below 1.0, and so it cannot be concluded that X rays would have no effect upon coincidences of small magnitude in this portion of the chromosome. But it can be concluded that coincidences of small magnitude in distal regions, and also all coincidences normally approximating 1.0, in any regions, are affected little or not at all by these doses of X rays.

DATA ON REACTION OF SECOND CHROMOSOME TO LIGHTER DOSE

The data relating to crossing-over frequency in this experiment have been analytically presented in table 5 and figure 6 in similar fashion to those of the preceding experiments. A glance at the figure or at the table, (especially at the line of quotients, which is the most informative) shows the remarkable similarity between the results here and in the experiment on the third chromosome in which the same dose was used. In the present experiment, as in the other, the only region distinctly affected was the centermost region (here *b-pr*), in spite of the fact that MAVOR, with his larger dose, found that the other region studied by him, *pr-c*, was affected

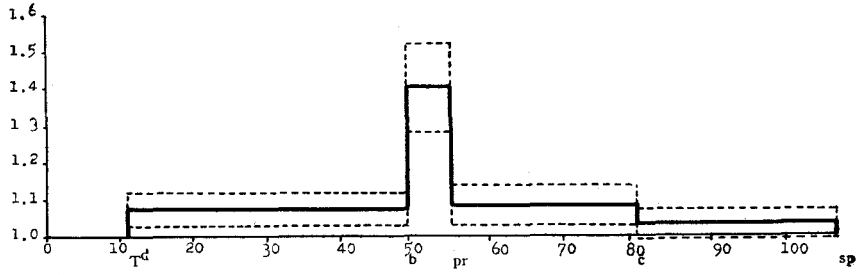


FIGURE 6.—Effect of lighter dose on chromosome II. Details as in figures 4 and 5.

also. A difference as large as that in the centermost region in this experiment would arise less often than once in 140 trials, if due purely to chance; differences as large as those in any of the other regions would arise oftener than once in 4 trials. The increase in the length of the total map,—6.4 units,—is also fairly significant, and about the same in amount as the increase (5.3) in the map length of chromosome III under the same conditions.

The coincidences for adjacent regions were calculated, and are given in table 6. As in the case of the corresponding coincidences in chromosome III, these are significantly lower than 1.0 for regions chiefly on the same side of the midpoint, but are approximately 1.0 for regions chiefly on opposite sides of the mid-point, even when these regions are adjacent and fairly short (2 and 3). This result agrees also with previous data on coincidence in chromosome II. Of greater interest is the fact that, as was

found to be the usual rule in chromosome III, there are no significant differences between the coincidences for adjacent regions in treated and control lots. (Coincidences for non-adjacent regions here were all within close range of 1.0, since in these cases the regions were always on opposite sides of the mid-point.) Again we must not conclude from this correspondence that in the central portion of the chromosome the smaller coincidences which might have been obtained from regions still closer together would not have shown significant differences.

The close parallel between the results for the second and the third chromosomes, when treated with the lighter dose, makes more significant the increase in crossing-over frequency in the central region of chromosome III, which by itself would not have seemed so decisive. Further, the marked difference between the reaction to the weaker and that to the

TABLE 6

Effect of lighter dose on coincidence in chromosome II.

ORIGIN OF DATA	REGIONS INVOLVED		
	1 and 2	2 and 3	3 and 4
Treated series, "T"	0.6±.076	1.2±.106	0.6±.049
Control series, "C"	0.8±.115	1.0±.13	0.7±.055
Difference, T−C	−0.2±.138	+0.2±.168	−0.1±.074
Difference÷probable error	1.45	1.2	1.36

stronger dose shown by the region next in proximity to the center acquires much greater significance from the fact that this same phenomenon appears both in the third chromosome (as previously noted) and in the second (as shown by comparison of the present results on p_r-c with those of MAVOR). The relative lack of effect of either dose on the frequency of crossing over in the distal regions is also made more certain by the correspondence of all the present experiments in this respect. That this apparent lack of effect, in these long regions, cannot be due to a relative increase in the proportion of double crossovers is proved by the coincidence studies.

DATA ON REACTION OF SECOND CHROMOSOME CONTAINING CROSSOVER-INHIBITORS TO LIGHTER DOSE

The chromosome bearing the gene for curly wings contained the crossover-inhibiting genes studied by WARD (1923),—one located in the left

half and preventing nearly all crossing over in this section, and the other in the right half, preventing most of the crossing over there. These effects occur in flies heterozygous for the genes (homozygous curly is lethal). The central region of the chromosome is less affected by either of these genes than the distal region in which the gene is located; thus even when both genes are present there is a small amount of crossing over between *b* and p_r, and between p_r and *c*. This is illustrated in the controls of the present experiment, on the second line of table 7, where it is shown that 2 crossovers were found in the first and 1 in the second of these regions, but none elsewhere, amongst the 2060 flies. Amongst the 901 flies from treated parents there were 9 crossovers altogether, 5 of these being in the most central and 4 in distal regions. Although the total numbers of

TABLE 7

Effect of lighter dose on chromosome II with curly present.

ORIGIN OF DATA	PERCENT CROSSOVERS IN TOTAL DISTANCE	TOTAL NUMBER COUNTED	NUMBER OF CROSSOVERS (ALL SINGLES) IN:				
			Region 1 T^d–b	Region 2 b–p_r	Region 3 p_r–c	Region 4 c–s_p	Total distance T^d–s_p
Treated series. "T"	1.0± .22	901	1	5	0	3	9
Control series, "C"	0.15± .06	2060	0	2	1	0	3
Difference of percents	0.85± .23						
Difference ÷ probable error	3.7						

crossovers are necessarily very small throughout, the difference between the total percents (map lengths) is significant, and it is decidedly probable, as well, that the percent of crossovers has been increased both in the center and also in the distal regions. If this is the case, it would seem that crossing-over frequency in the distal regions is more readily affected by X rays when genes that interfere with crossing over are present. In the central region X rays affect crossing over readily both in the presence or in the absence of such genes.

As to the degree of the effect produced, the quotient of treated total map length divided by control is 6.9, but this quotient has little meaning for the making of comparisons with the quotients obtained in the other experiments, since the absolute numbers of crossovers obtained here were so

small that the probable error, ± 3.1, of the above quotient, is very large in proportion to its own value. Certainly, however, the total length is affected more here, proportionately, than in the other experiments, since in them the bulk of the length was made up of the unaffected distal regions. There is no question of possible effects of change in double-crossover frequency upon the total length in the present experiment, as no double crossovers occurred either in treated or in control sets.

THE STATISTICAL TREATMENT

In calculating the probable errors, the customary procedure was followed, of regarding each sample as a random one, aside from possible differences due to the controlled element of the treatment (X rays). Any deviations greater than would commonly be found between random samples were, therefore, held to indicate an effect due to treatment. Such is the method which has been followed by MAVOR and by most other geneticists in handling their data.

The fact should not be lost sight of, however, that there may possibly be other, uncontrolled sources of difference between the results in "treated" and "control" series, such as differing composition with regard to "invisible" genes, or with regard to unnoticed or uncontrollable environic conditions to which the parents or batches of offspring may at some time have been subjected. As the parents are divided at random between treated and control series, and no distinction is made in the method of handling of the two series of cultures, such differences would, of course, have an equal chance of existing amongst the cultures of either treated or control series alone, but their random distribution among the members of the two series would add another source of deviation between the two final results, in addition to the effect of the treatment, and to the random sampling of the germ cells which the final counts of each culture represent. GOWEN's (1919) statistical studies on crossing over have shown that the frequency of this event is often so variable, from culture to culture, that we must assume the comparatively frequent occurrence here of "invisible" influences like those mentioned.

Fortunately, the effect of such influences may be gauged in any experiment in which a number of cultures (that is, sets of samples) have been counted in both control and treated series. If such influences are not at work, the cultures in either series, separately, will not differ from each other more than random sets of samples should,—a matter not difficult to determine by comparing the standard deviation of the values in the cultures with the standard error to be expected of sets of random samples

of a size equal to the harmonic mean size of the actual cultures. In that case, then, the ordinary formulae for random sampling may be used in determining the "errors" of control and treated series, and of their differences and quotients, and departures sufficiently exceeding these "errors" may be taken as proving an effect produced by the treatment. If, however, the cultures of either treated or control series prove to differ more amongst themselves than random sampling would reasonably allow, a different method must be followed. In this event, the standard deviation (or "probable deviation") which these cultures are observed to show amongst themselves (in regard to the character considered, such as crossover frequency) must be divided by the square root of the number of cultures forming the series. The resulting value must be used as the standard, or "probable," deviation of the value of this character for the series as a whole (treated or control series, as the case may be), and any theoretical "probable error," calculated on the assumption of purely random samples, will not apply. The value obtained as above is, however, itself subject to considerable (though calculable) inaccuracy, unless the number of cultures in the series is large.

In the case of the present data a considerable number of comparisons were made between the standard deviations shown amongst the cultures of a given series, in regard to particular crossover frequencies, and the standard deviations, or "standard errors," to be expected if these cultures really represented random samples drawn from the same material. The correspondence was close throughout, the differences between observed and calculated standard deviations being within the limits of error to be allowed for the calculated standard deviations themselves. It was thus evident that the use of the ordinary formulae of random sampling would here be permissible for determining the significance of the results, and it was also apparent that since the data from a given series were in this sense homogeneous, they might all be added together, for presentation in condensed form, instead of being shown by individual tabulation of the results concerning all 64 classes of each bottle. Finally, a pragmatic test of the significance of the data for determining the effects of the agent under control (X rays), as contrasted with uncontrolled influences, was forthcoming, when the results of the different experiments were compared and found mutually consistent, the results on the two chromosomes paralleling each other in a way that would be most remarkable if due to unregulated agents.

Another customary, but dubious, statistical practice of geneticists, which was followed here, was the calculation of the probable errors by a formula

which really gave the deviations to which other observed values would have been subject, if the given observed value were itself precisely equal to the true value in the material as a whole. As a matter of fact, however, the true value may be either smaller or larger than that observed, and what is really wanted is the probable deviation that observed values would show, firstly, on the assumption of the largest true value that might "reasonably" have been expected to give the value originally observed, and, secondly, on the assumption of the smallest "reasonably possible" true value. As the larger extreme hypothetical true value would have a somewhat larger error than a true value equal to that observed, and the smaller extreme hypothetical true value a smaller error, the figures that are really desired differ somewhat from the probable errors as ordinarily found. The discrepancy is more pronounced when the values considered are themselves small in proportion to their probable errors. Serious miscalculations are seldom caused in this way, however, and rigorous methods for determining the more strictly accurate values do not seem to have been well worked out. It was not considered worth while, in the present work, to go through the laborious and sometimes doubtful calculations that the use of approximation methods for this purpose would have entailed. This slight source of inaccuracy, then, which is common to nearly all statistical work, applies throughout the preceding and the following calculations, in the case of all probable errors employed,—including those for crossover frequencies, map lengths, differences and quotients.

The value termed "probable error," as here used, is indirectly determined and approximate, as the value first calculated in each case is the "standard error," which is multiplied by .6745 to obtain the supposed "probable error." Standard error, unmodified, might be a better measure of deviations, but "probable error" has been employed in the present tables because it is more familiar to the average biological reader, who is accustomed to seeing it appended to an observed value, after a $\pm$ sign, without any further notation being necessary.

The formula used to find the standard error of the crossover frequency (E_p) was the familiar one $E_p = \sqrt{\frac{p(1-p)}{n}}$, where p = proportion of cross overs (observed) and n = total number of individuals in the count. For the standard error of the map length (E_m) a new formula had to be worked out, the derivation of which is given in a paper which will appear in the November issue of GENETICS; this formula is $E_m = \sqrt{\frac{m(1-m)+2\cdot1d+3\cdot2t+4\cdot3q+}{n}}$,

where m represents the total map length (in proportions, i.e., in "chromosome units" divided by 100), d = the proportion of double crossovers, t the proportion of triples, q of quadruples, etc.

The standard error of coincidence (E_c) also presented a problem not hitherto solved. The solution of this, also explained in the parallel paper, is as follows:

$E_c = c\sqrt{\frac{1-c(a_s+b_s+ab)}{d\,n}}$, where c is the coincidence, a and b are the proportions of (all) crossovers in the first and second regions considered, respectively, and a_s and b_s the proportions of single crossovers in each of these regions (or at least crossovers not involving double crossing over occurring in these two regions simultaneously). As explained in the section on "partial coincidence," n may, if non-adjacent regions are considered, be limited to the number of those individuals not having any crossing over in an intermediate region; in that case a, b, d, a_s and b_s are correspondingly limited, and are calculated on the basis of the limited n; the formula still holds, however.

For the standard error of a difference (E_d) the common formula, $E_d = \sqrt{\sigma_1^2+\sigma_2^2}$, was used, where σ_1 and σ_2 are the standard deviations of the two quantities whose difference is being considered. For the standard error of a quotient (E_Q) the formula $E_Q = Q\sqrt{\left(\frac{\sigma_1}{n_1}\right)^2+\left(\frac{\sigma_2}{n_2}\right)^2}$ was employed, Q being the quotient itself, n_1 the value forming the numerator and n_2 that forming the denominator of the quotient, and σ_1 and σ_2 being the respective standard deviations of the latter. Like the formula for the difference, this formula only holds provided the two values entering into it are uncorrelated, but this must of course be true in the present problems.

For some reason, quotients seem seldom used in genetic statistics, and their probable or standard errors are very rarely seen, comparisons being made almost exclusively by means of differences. There are many problems, however, the solution of which requires the use of quotients and their errors. In the present work, the quotients of treated over control, and their errors, are necessary for the main purpose,—a comparison of the intensity of the effect of treatment in the different regions. The corresponding differences between treated and control in the different regions cannot be used for this comparison, since the size of the difference obviously depends not only upon the intensity of the effect, but also upon the size of the region considered. We may now make an examination of these quotients, in a final analysis of the results of the

TABLE 8

Differences between effects of X rays on "susceptible" and other regions. (Effects measured in terms of quotients ($T \div C$) of treated divided by control frequencies.)

CHROMOSOME III, LIGHTER DOSE, CENTRAL AND OTHER REGIONS			CHROMOSOME II, LIGHTER DOSE, CENTRAL AND OTHER REGIONS			CHROMOSOME III, HEAVIER DOSE, CENTRAL AND OTHER REGIONS			CHROMOSOME III, HEAVIER DOSE, SUBCENTRAL AND OTHER REGIONS		
Regions involved	Difference between quotients	Diff. ÷P.E.	Regions involved	Difference between quotients	Diff. ÷P.E.	Regions involved	Difference between quotients	Diff. ÷P.E.	Regions involved	Difference between quotients	Diff. ÷P.E.
3–4	0.53 ± .23	2.1	2–3	0.33 ± .13	2.5	3–4	0.2 ± .35	0.57	4–3	−0.2 ± .35	0.57
3–2	0.46 ± .22	2.1	2–1	0.33 ± .13	2.5	3–2	0.88 ± .30	3.0	4–2	0.68 ± .20	3.4
3–5	0.47 ± .23	2.0	2–4	0.37 ± .125	3.0	3–5	1.05 ± .30	3.5	4–5	0.85 ± .20	4.2
3–1	0.49 ± .22	2.2				3–1	1.11 ± .29	3.8	4–1	0.91 ± .20	4.6
3–rest	0.49 ± .22	2.3	2–rest	0.34 ± .12	2.8	3–rest	0.81 ± .31	2.6	4–rest	0.66 ± .25	2.7

foregoing experiments, and we may use the errors of these quotients to determine the degree of certainty of the final conclusions.

FINAL ANALYSIS

Reference to the next-to-the-last lines of tables 3 and 5 shows that in the central region a significant or probably significant difference between control and treated was produced in each case (chance 1 in 20 in chromosome III, 1 in 200 in chromosome II, for such a difference to be an "error"), and that in none of the other regions were significant differences produced. This by itself, however, does not prove the thesis that the X rays affected the central region without affecting the distal regions, or even that they affected the center more than the distal parts; for the possibility would still remain that the X rays really affected all regions equally but weakly, if the center had chanced to be a plus random variate and the other regions minus random variates of one effect. To eliminate this possibility, and thus prove a differential effect of the rays, it is necessary to show that the intensities of effect, represented by the quotients, for the center, are significantly larger than those for the other sections. Hence, we are now required to obtain the differences between the quotients for the various regions, and examine the significance of these quotient-differences by comparing them with their own "probable errors." The latter are, of course, found by the formula $E_{dQ} = \sqrt{E_{Q_1}{}^2 + E_{Q_2}{}^2}$.

A list of the differences between the quotient for the center and the quotient for each of the other regions, together with their probable errors and the ratio they form to the latter, in the case of each of the first three experiments, is given in table 8. In the experiment involving chromosome III and the lighter dose, reported in the first column of the table, it is seen that the difference between the quotient for the center and the other quotient in each case hovers about 2.1 times its probable error. Each of these differences by itself is, therefore, not very significant. The differences between the quotient for the center and the mean of the quotients for the rest of the chromosome is given on the last line. The probable error of this mean value is obtained by calculating the square root of the sum of the squares of the probable errors of the individual values, and dividing the result by the number of these individual values (in this case 4). It will be seen that the difference between the quotient for the center and the mean of the other quotients is 2.3 times its probable error; thus, it is slightly more significant than the individual differences, representing a chance of 1 in 9.5 if there were really no difference between the quotients.

Turning now to the parallel experiment on chromosome II, (2nd column) we find that the quotient for the center differs from each of the others by values ranging from 2.5 to 3.0 times the probable error (representing chances of 1 in 14 to 1 in 23 on the assumption of no real positive difference). The difference in quotients between the center and the mean of the rest of the chromosome is 2.8 times its probable error, which represents a chance of 1 in 17 if the true values were equal (or if the quotient for the center were really smaller).

In each of the above experiments, separately, then, the probability is only moderate that the center was affected by X rays more than the distal regions. We may, however, combine the results from the two experiments, asking the question, what is the probability that in both of them together (i.e., in at least one of the two experiments) the X rays affected the middle more than the ends? To answer this question we may take the mean of the differences between the quotients for the center and for the rest of the chromosome (as a whole) in the two experiments; this value is 0.414. Its probable error (obtained like the error of the means in the preceding pages) is 0.133. Hence the mean difference is 3.1 times its own probable error, which represents a chance of 40 to 1 that the mean difference is significant. We may therefore conclude that it is really very probable that X rays have produced a differential effect upon the center as contrasted with the remainder of the chromosome as a whole, in at least one of the experiments with the lighter dose of rays, and, since there are *a priori* reasons for believing that the two chromosomes would be affected similarly (as well as the argument from the present similarity of data) it becomes extremely likely that both chromosomes were affected in this way.

By following an identical method it may be shown that the chance is 33 to 1 that the central region has in both experiments been affected more markedly by the light dose of X rays than the region (p_r-c or p-s_s) just "to the right" of it, and next in proximity to the center. The calculations involving the center and each of the more distal regions, considered individually, give in every case a considerably higher probability than this. Our original conclusion concerning differential action on the center as contrasted with the rest of the chromosome as a whole may accordingly be extended to each of the regions in this remainder of the chromosome, individually.

The experiment on the third chromosome, involving the stronger dose, proved decisively the effect of X rays on both the region including the central point and that next in proximity to the latter, and failed to show an effect on the distal regions. We cannot, however, conclude that there

was really a weaker effect (much less that there was no effect) on the distal regions until we determine whether the differences between their quotients and those for the more central regions are significant. The third column of table 8 shows that the differences between the quotients for the central and for each of the "distal" regions range from 3.0 to 3.8 times their probable errors (according to the distance of the latter regions from the center), giving chances of only 1 in 20 to 1 in over 100 that these differences have no significance. The differences between the quotients for the p-s_s region and the other regions, with their probable errors, are given in the fourth column of the table. In this case the differences are even more significant than those for the central region (owing to the greater accuracy of the count for the larger p-s_s region); they range from 3.4 to 4.6 times their probable errors (chances 1 in 45 to 1 in 500).

TABLE 9

Effects of heavier dose on composite regions of chromosome III.

SECTION	TREATED FREQUENCY	CONTROL FREQUENCY	QUOTIENT (T÷C)	DIFFERENCE BETWEEN QUOTIENTS	DIFFERENCE ÷PROBABLE ERROR
r_u-s_t	40.8±.87	41.1±1.19	0.99±.036	0.89±.152	5.9
s_t-s_s	16.9±.65	9.0±.62	1.88±.148		
s_s-e	10.8±.52	11.4±.74	0.95±.076	0.93±.166	5.6

The most decisive manner of handling the data in the above experiment is to compare the quotient for the entire central section from s_t to s_s (including s_t-p and p-s_s) with that for the entire left distal section, r_u to s_t (including r_u-h and h-s_t), and with that for the right distal region, s_s-e. The errors of the quotients are worked out as before, but can now be based on the errors of the control and treated values for composite regions; the latter errors are obtained by the use of the formula previously given for the standard error of a "map length." The quotients and their errors, so obtained, together with the results concerning the differences of these quotients, are given in table 9. The more central "composite region," or "section," differs from the distal section by 5.85 times the probable error of this difference and from the right distal region by 5.6 times the error of the latter difference; the first set of quantities gives a chance of less than 1 in 7000 and the second set a chance of less than 1 in 12,000 that the intensity of the X-ray effect is not greater on the more central region. The differential effects in this experiment, then, are the most conclusively

established of all,—which is in accordance with the fact that a stronger dose was here used.

The results of this experiment with a strong dose differ from those obtained with a lighter dose, first, in that they are more pronounced and decisive, and second, in that they indicate an effect of the X rays over a greater length of the more central region. We may now inquire whether the differences between the effects of stronger and weaker doses are themselves "significant," or whether they could result from random sampling.

Considering first the third chromosome, we find that in the case of the most nearly central region, s_t-p, the difference between the quotient for treated over control with the light dose, $1.52 \pm .21$, and the corresponding quotient with the heavy dose, $2.0 \pm .29$, is $0.48 \pm .36$. As this is only 1.3 times its own probable error it has by itself very little significance. In the case, however, of the subcentral region of this chromosome, p-s_s, there is a difference of $0.81 \pm .21$ between the quotient for the light dose, $0.99 \pm .093$, and that for the heavy dose $1.8 \pm .189$. This difference, being 3.85 times its probable error, would occur but once in more than a hundred trials, if there were really no difference between the effects of the two doses. Thus, it is practically certain that the heavier dose affected at least a part of the s_t-s_s section more strongly than did the lighter dose. While it is quite possible that within this section the subcentral region, p-s_s, has its crossing-over frequency more raised by a change from light to heavy dose than does the centralmost region, s_t-p, (relatively to the effect which the light dose itself causes), nevertheless, the errors are much too large to make sure that there is a real difference between the two regions in this respect.

Turning now to the second chromosome, and considering first the centralmost region, b-p_r, we find that for the lighter dose the treated-over-control quotient is $1.4 \pm .12$. For the stronger dosage we may use MAVOR's data, relating to flies from eggs laid 6 to 12 days after treatment with 65 H (averaging together the results from 62.4 H and 68 H). From these data we may compute, by the methods outlined in the present paper, a quotient of $4.07 \pm .315$ for the b-p_r region. The difference between the quotients for the two doses here, then, is $2.67 \pm .34$. This is 7 to 8 times its probable error, and hence unquestionably significant. On the other hand, for the subcentral region, p_r-c, the data of the present paper, involving the lighter dose, give a quotient of $1.08 \pm .053$, and MAVOR's data, for the heavier dose, may be reckoned as giving $1.23 \pm .055$. The difference here is $0.15 \pm .076$. This would represent a chance of 1 in 5.6 on the assumption of no real difference in effects of the two doses, but about the same chance if the

heavier dose really produced as much increase in crossing over, relatively to the effect of the lighter dose, as in the case of b-p_r. For the second chromosome, then, we find an undoubtedly stronger effect on the central section as a whole, b-c, in MAVOR's experiments than in those of the present paper, but we cannot tell whether the two "regions" of this section are affected to the same extent (relatively to the effect caused by the lighter dose) by a change from lighter to heavier dose.

There is no real ground in the above results, then, for assuming a difference between the second and third chromosomes, in regard to the chief seat of effect within the central section, of an increase in dosage, even though the raw data suggest such a possibility, and this assumption would, if anything, seem rather unlikely, in view of the similarity in behavior otherwise shown by these chromosomes, both in the present and in other experiments. Neither can we, in the case of either chromosome, decide from the present data whether the lighter dose of X rays is really more localized in its place of action than the heavier dose. For, although the lighter dose appeared to produce no effect whatever on the subcentral region of either chromosome, the errors were great enough to allow for an effect as large, in proportion to the effect of the same dose on the central-most region, as that found in the case of the heavier dose. All that can be said is that not until the heavier dose was used were the effects on the subcentral regions pronounced enough to be detected under the conditions of the experiment.

In the case of the second chromosome, there is an additional chance for error in comparing the effect of the two doses, inasmuch as (1) MAVOR's work was done on a stock of widely different origin from that used by the present writer, and (2) we do not know whether the individual cultures in MAVOR's experiment really represented random samples, or varied more than random samples from one another, due to environic or genetic causes. The latter suggestion seems to receive a certain amount of support in the fact that when the results obtained by MAVOR with 62.4 H and 68 H are compared, it turns out that the quotient of treated over control for b-p_r was higher with the latter dose, but the quotient for p_r-c was higher with the former dose.

We may consider, lastly, the question of possible reversal of the effect of X rays on crossing-over frequency, with change of dosage. This was originally suggested by PLOUGH (1924), chiefly in order to reconcile MAVOR's findings of a decrease in crossing-over frequency in chromosome I, and an increase in chromosome II, when similar doses of X rays were applied. PLOUGH's suggestion was that weaker doses of X rays may cause

a decrease and stronger doses an increase of crossing-over frequency, in the same chromosome (or, we may now say, "chromosome region"); that chromosomes (or "regions") may, however, be different in their susceptibility to X-ray influence; and that, therefore, the same dose of rays may cause a decrease in crossing-over frequency in one portion of the chromatin, and an increase in another portion, which is more susceptible to their action.

In the present experiment, even when considered in connection with MAVOR's results on chromosome II, there is no case of a decrease in crossing over with lighter dose, followed by an increase with stronger dose, in any of the regions studied in either second or third chromosome. If such an effect can be produced on the central region or on the one next in nearness to the center, then both these regions must be so very susceptible to X rays that the critical amount, beyond which a decrease of crossing over is not produced, had already been attained even by the lightest dose used. On the other hand, if such a reversal of effect can be produced on the distal regions of chromosomes II and III, then these regions must be so insusceptible that this lighter dose did not have sufficient strength to cause even a decrease of crossing over in them. With the heavier dose, the data did show a slight decrease in the two distalmost regions studied in chromosome III (there being no data available for the effect of the heavier dose on distal regions of chromosome II), but the decrease was only 2.1 and 0.7 times its probable error in the two respective cases. The average decrease in these two regions taken together was 1.55 ($\pm$.75) percent; this difference, being 2.1 times its probable error, would occur once in seven random samples even if the decrease were not real. The result, therefore, by no means proves a decrease in these regions, and if there is a decrease it is much less than that found by MAVOR in chromosome I. Even if taken at its face value, however, there is nothing to show that a still heavier dose would cause an increase of crossing over in these regions.

If the central regions are so very susceptible and the distal regions so very insusceptible to the X rays, it might be expected that in some intermediate region that was studied,—such as h-s_t or T^a-b,—there would be an intermediate susceptibility, such that one of the two doses used, at least,—either the lighter or the stronger one,—would cause a noticeable decrease of crossing over. Such an intermediate susceptibility would be especially to be expected in view of the proved susceptibility gradient in the central regions (the centralmost region having been affected significantly more than the adjacent one, by the lighter dose). There is,

however, no sign of such a crossing-over decrease in the "intermediate" regions. Instead, there are doubtful, very slight, increases of crossing over in these regions, especially with the stronger dose. It is difficult to reconcile this result with the idea of a reversible action with increase in dosage, unless we suppose a peculiar curve of susceptibility along the length of the chromosome. The curve, after presumably falling off gradually from a maximal height at the center, would have suddenly to fall off very sharply, at some intermediate distance, to a level very far below that for the center. While such a situation would be by no means impossible, it must be admitted that it would involve rather special and peculiar conditions, and these special requirements of the hypothesis of a reversible effect of the X rays would argue against the probability of that hypothesis.

SUMMARY

1. The comparatively light X-ray dose of 26.8 Holzknecht units causes a significant increase in the amount of crossing over in the central regions of both the second and third chromosomes (the long V-shaped autosomes) of *Drosophila melanogaster*. In the case of both chromosomes the region so affected is (normally) not much over 6 units long; "subcentral" regions adjacent to this, as well as distal regions, have their crossing-over frequency affected little or not at all by the above dose.

2. a. Application of twice as strong a dose of X rays, in the case of the third chromosome, causes the frequency of crossing over to be significantly above that in the controls not only in the central region but also in the "subcentral" region,—in which, moreover, the difference between the results with the lighter and heavier doses is significant likewise. More distal regions, however, have their crossing-over frequency sensibly unaffected (or possibly very slightly decreased) even by the stronger dose.

b. For the second chromosome, the results of the present experiments with the light dose may be compared to MAVOR's results for black-purple-curved, in which a relatively heavy dose was used. Again we find a significantly stronger effect from the heavier dose, and a significant effect upon the "subcentral" region with this dose only, but data are not available to show the effect of the heavier dose upon the distal regions of this chromosome.

3. The above results prove that there exists in both long autosomes a regionally differential susceptibility to X rays, with the maximum susceptibility in the morphologically differentiated portion at and near the bend of the V. The effect of the X rays is not confined to a single central point however. These intrachromosomal differences in susceptibility are

very great, being comparable in size to the interchromosomal differences found by MAVOR between certain regions in the first and second chromosomes.

4. In view of these conclusions, it will be seen that adequate comparisons of the effects on different chromosomes can be made only after various sections of them, representative of the different kinds of regions comprising them, have been studied. The second and third chromosomes, when studied in this manner, prove to be very similar in their reactions.

5. No evidence has appeared in these experiments for the idea that the X-ray effects may be reversed in sign with change in dosage,—that is, that weaker doses may cause a decrease, and stronger doses an increase of crossing-over frequency, in a given section of chromatin. It is shown that if the idea is correct, adjacent regions in an autosome must differ very sharply in susceptibility.

6. Coincidence of crossing over, so far as it could be studied in these experiments, was affected little, if at all, in either the second or the third chromosomes, by the doses of X rays here used. This result applies to coincidences below 1.0 occurring in distal regions, and coincidences about 1.0, involving either distal or central regions, or both, but central regions short enough and close enough together to give coincidences below 1.0 were not available for study.

7. Even when the crossing-over-inhibiting genes carried in the chromosomes containing "curly" are present in one of the second chromosomes, application of X rays causes an increase of crossing-over frequency. This increase is again noticeable in the central region, but it probably occurs also in the distal regions in this case.

8. Difficulties involved in obtaining the records,—which required the classification of each of the 10,439 flies counted into one of 32 or 64 possible combinations,—were reduced by means of a special method of tabulation, presented in the text, which can be used to advantage in the case of all complex linkage counts.

9. In the statistical analysis of the data, new methods and formulae are employed, which are also applicable to other genetical work.

LITERATURE CITED

BRIDGES, C. B., 1915 A linkage variation in Drosophila. Jour. Exp. Zoöl. **19**: 1-21.

1921 Revised map of the third chromosome of Drosophila. Unpublished.

BRIDGES, C. B., and MORGAN, T. H., 1919 Contributions to the genetics of *Drosophila melanogaster*. II. The second-chromosome group of mutant characters. Carnegie Inst. Washington Publ. 278, pp. 123-304.

1923 The third-chromosome group of mutant characters of *Drosophila melanogaster*. Carnegie Inst. Washington Publ. 327. 251 pp.

GOWEN, J. W., 1919 A biometrical study of crossing over. On the mechanism of crossing over in the third chromosome of *Drosophila melanogaster*. Genetics 4: 205-250.

MAVOR, J. W., 1923 An effect of X rays on the linkage of Mendelian characters in the first chromosome of Drosophila. Genetics 8: 355-366.

MAVOR, J. W., and SVENSON, H. K., 1924 An effect of X rays on the linkage of Mendelian characters in the second chromosome of *Drosophila melanogaster*. Genetics 9: 70-87.

MULLER, H. J., 1916 The mechanism of crossing over. Amer. Nat. 50: 193-221, 284-305, 350-366, 421-434.

1920 A quantitative study of mutation in the second chromosome of Drosophila. Paper read before the American Society of Naturalists, December 1920; Unpublished.

PLOUGH, H. H., 1917 The effect of temperature on crossing over in Drosophila. Jour. Exp. Zoöl. 24: 147-209.

1921 Further studies on the effect of temperature on crossing over. Jour. Exp. Zoöl. 32: 187-202.

1924 Radium radiations and crossing over. Amer. Nat. 58: 85-87.

STURTEVANT, A. H., 1919 Contributions to the genetics of *Drosophila melanogaster*. III. Inherited linkage variations in the second chromosome. Carnegie Inst. Washington Publ. 278, pp. 307-341.

WARD, L., 1923 The genetics of curly wing in Drosophila, another case of balanced lethal factors. Genetics 8: 276-300.

WEINSTEIN, ALEXANDER, 1918 Coincidence of crossing over in *Drosophila melanogaster* (*ampelophila*). Genetics 3: 135-172.