AN ANALYTICAL STUDY OF CROSSING OVER IN *DROSOPHILA MELANOGASTER*

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Received March 7, 1935

INTRODUCTION

In 1902, SUTTON pointed out the close analogy between Mendelian heredity and the cytological processes of chromosome reduction and fertilization. He clearly foresaw and predicted the discovery of linkage groups (1903). The discovery of linkage (BATESON, SAUNDERS AND PUNNETT 1906) and the formulation of the chiasmatype hypothesis (JANSSENS 1909) followed shortly thereafter. When linkage was discovered and analyzed in the sex chromosome of *Drosophila* in 1910, it was interpreted by MORGAN and his students in terms of the chiasmatype hypothesis (MORGAN 1911, MORGAN, STURTEVANT, MULLER and BRIDGES 1915, MULLER 1916).

With the accumulation of genetic data on *Drosophila* and other forms, additional concepts regarding the physical basis of linkage were developed. Chief among these were: (1) the linear order of the gene, (2) interchange between homologous chromosomes in blocks of genes, (3) interference, (4) chromosome maps, indicating the location of genes with respect to each other, and (5) the limitation of linkage groups.

With the discovery of double-strand (four strand) crossing over (BRIDGES 1916), the analysis of linkage data in attached X's (ANDERSON 1925, L. V. MORGAN 1925, STURTEVANT 1931, RHOADES 1931, EMERSON and BEADLE 1933), triploids (BRIDGES and ANDERSON 1925, REDFIELD 1930, 1932) and heteroploids generally, translocations, and with analysis of X-ray effects on mutation, etc., it has been recognized for some years (MORGAN, BRIDGES and STURTEVANT 1925, MULLER 1930, MULLER and PAINTER 1932, DOBZHANSKY 1930, 1931, 1932a, b, 1933, REDFIELD 1930, 1932, BRIDGES 1932a) that analyses, in terms of the leading concepts regarding the mechanism of crossing over, describe these phenomena only approximately. Recently, several workers (WEINSTEIN 1932, MATHER 1933, RHOADES 1933, EMERSON and RHOADES 1933, GRAUBARD 1934, SCHWEITZER 1934) have called attention to some theoretical changes that have become necessary in order to describe more adequately the recorded data on recombination among linked genes.

In this paper, some of the linkage data from *Drosophila melanogaster* will be analyzed with the object of elucidating certain further details of the cytological processes involved.

Many friends and co-workers have rendered assistance on numerous

occasions with the computations and interpretations, on which the following is based. For this the author wishes to make acknowledgement here. He is especially indebted to Mrs. L. V. MORGAN of the CALIFORNIA INSTITUTE OF TECHNOLOGY for permission to make use of data prior to publication, and to her and to Professor A. H. STURTEVANT of the CALIFORNIA INSTITUTE OF TECHNOLOGY, Doctor M. A. GRAUBARD and Professors D. E. LANCEFIELD and L. C. DUNN of COLUMBIA UNIVERSITY, for many helpful suggestions in connection with the following work.

THE POSTULATES OF CROSSING OVER

Ambiguity has arisen from the confused usage of the words recombination and crossing over, which are occasionally used as synonyms and at other times as distinct and different terms. BRIDGES has recently suggested (1932a) that the term crossing over should refer to the cytological mechanism, whereas recombination has reference to the empirical results obtained from an F_2 or a backcross. The procedure that will be followed in this paper will be to refer to the cytological events as crossing over and to the genetic results as recombination of linked genes.

Several of the concepts referred to have been sufficiently verified and require little additional discussion.

1. The work of STERN 1931, CREIGHTON and MCCLINTOCK 1931, and RHOADES 1932, demonstrates that genetic recombination is accompanied by cytological crossing over.

2. Extensive analyses of recombination data have shown that the exchange of genes between homologous chromosomes does indeed occur in blocks (STURTEVANT 1913, 1915, MORGAN, BRIDGES and STURTEVANT 1925).

3. The recent findings with induced translocations and deficiencies verify the linear order of the genes, as postulated (MULLER and PAINTER 1929, DOBZHANSKY 1929).

4. The correspondence between the number of chromosomes and the number of linkage groups has also been established in the forms most thoroughly investigated (see especially MORGAN, BRIDGES and STURTEVANT 1925, STERN 1927, R. A. EMERSON 1932, etc.).

Our understanding of the chromosome maps and our concepts of coincidence and interference need to be modified, however, in order for them to continue to be useful in elucidating the mechanism of crossing over from data on the recombination of linked genes.

COINCIDENCE AND INTERFERENCE

Recombinations involving two regions in a chromosome simultaneously generally occur less frequently than are to be expected from the known

incidence of recombination in each region separately. The comparison between the number obtained and those expected has been expressed as a ratio obtained/expected, which is known as the coincidence ratio or index. The cytological events which apparently underly the variable coincidence index may be stated as follows: simultaneous double (and multiple) recombinations occur less frequently than are to be expected on a chance basis in nearby regions and are progressively less frequent as the distance between the breaks diminishes. This relation has usually been expressed as the interference of one cross over on the occurrence of another nearby.

Certain difficulties have appeared in the use of the coincidence index as a measure of interference:

(1) Coincidence values differ in certain critical cases depending on whether the calculations are made as complete coincidence (MULLER 1916) or as partial coincidence (WEINSTEIN 1918). This has recently been indicated by WEINSTEIN (1932) and by the present writer (1934).

(2) The indices obtained when computed for two regions with fairly long, intermediate genetic distances will differ when calculated from different sets of data depending on the number of *undetected* double crossovers that occurred within the limits of the regions being considered in the particular experiment from which the computations are made (SCHWEITZER, loc. cit.).

(3) No simple theoretical interpretation of coincidence can be given because of the way in which the data are grouped. The usual procedure is to lump together in the non-crossover class all flies which do not give recombination in at least one of the two regions under consideration. For example, in a cross involving genes $a\ b\ c\ d\ e\ f\ g$ (denoting the regions by numbers, that is, $a-b=1$, $b-c=2$, etc.) to calculate coincidence for $a-b$ and $d-e$ (that is, 1, 4) all the 2, 3, 5, and 6 flies are treated as non-crossovers in the data. Similarly, 1, 2 flies are treated as 1, etc. and 1, 4, 6 flies are grouped with the 1, 4 class. But it is known that recombination in regions 2, 3, 5 or 6 do themselves restrict the incidence of simultaneous exchanges in region 1 and region 4. Consequently, coincidence, as measured, bears no simple relation to the cytological forces of interference of which it (coincidence) is a result.

In my previous paper, a method for the direct expression of interference relations between any two regions is given. When computed by this method, only those classes having direct reference to the events in the two particular regions being studied are included. In the example previously cited, classes 2, 3, 5 and 6 and all multiples except 1, 4 are excluded entirely from consideration. Only four classes are used, the non-crossover class, the class giving recombination for region 1 only, the class giving recombination for region 4 only, and the double crossover class 1, 4. The ratio calculated is called *fraction realized*.

RECOMBINATION VALUES AND CHROMOSOME MAPS

The construction of genetic chromosome maps

The construction of chromosome maps with numerical values has been in units of recombination percent. The distance between two genes on a chromosome has been defined as the sum of the recombination percents of the loci included between them, taken two at a time. For example, for genes $a\ b\ c\ d\ e\ f\ g$, the distance $a-g$ is equal to the sum of $a+b$, and $b+c$, etc. . . . and $f+g$ (BRIDGES and MORGAN 1923).

It was recognized and stated when even the earliest genetic maps of the X chromosome of *Drosophila* were made by STURTEVANT in 1913, that these maps did not necessarily accurately represent the spatial relations of the genes (STURTEVANT 1913, MORGAN, STURTEVANT, MULLER and BRIDGES 1915), and that any influence that would tend to modify the recombination values would thereby change the positions of the relevant loci on the maps, and by the same amount. It has been considered likely that the different degrees of crowding of genes that appear in different regions of the maps represented differences in crossover values of different parts of the chromosome, rather than differences in mutation rate, etc. (MORGAN, BRIDGES and STURTEVANT 1925, page 91 on, DOBZHANSKY 1929).

Chromosome maps and the cytological alignment of genes

In the tetrad, three perhaps independent sets of conditions are involved, of which recombination of linked genes is observed as the end result. These are synapsis, exchange and disjunction. Changes in any one will affect recombination values. When crossing over is discussed, the reference is apparently usually intended to refer to the mechanism of exchange, and all differences in percentage of recombination are referred to the proximity of the spindle fibre or to the distances between the loci, these two factors having been primarily considered as responsible for differences in recombination. In addition, in a form like *Drosophila*, the normal recovery of only one chromatid of a given tetrad must be taken into account. While it is true that every case of recombination of linked genes represents an antecedent event of exchange, every single or multiple exchange in a tetrad does not necessarily yield an offspring with an equal number of recombinations. This is so because only two of the four strands are involved at any level and only one strand of any tetrad is normally recovered, and furthermore, there may be a correlation between exchange and disjunction (WEINSTEIN 1932, R. EMERSON and RHOADES 1933, S. EMERSON and BEADLE 1933, L. V. MORGAN 1933, MORGAN, BRIDGES and SCHULTZ 1933). It may be accepted, therefore, that there is not a direct and simple relation between the genetic maps and the spatial arrangement of the genes.

The results of several lines of investigation, and especially the study of cytological configurations derived from translocations induced by X-rays (PAINTER and MULLER 1930, 1932, MULLER and ALTENBERG 1930, DOBZHANSKY 1930a, 1931) have supported these doubts regarding the accuracy of the distances on the genetic chromosome maps.

Recently, several new types of maps of the chromosomes of *Drosophila* have been published, differing in certain respects from the old maps. (1) REDFIELD has published a genetic map of the II and III chromosomes, based on data from triploids. These show less crowding of the genes than the ordinary genetic maps, but being similarly derived, these maps have the same general weaknesses as the old maps. In addition, the comparison between diploid and triploid maps is inadequate, because in the triploid offspring only one strand from a hexad is normally recovered, while in the offspring of diploids one quarter of the tetrad strands are obtained. In the triploid also, the possible frequent changes of partner in synapsis must be taken into account when comparisons are made with the diploid. (2) DOBZHANSKY (1929, 1930, 1931, 1932) and PAINTER and MULLER (1929a, b, 1932) have published approximate maps based on studies of metaphase plates involving fragments and translocations obtained in X-ray experiments. These maps probably represent a closer approximation to the pre-crossing over condition, but there are certain defects in their construction which render them unsuitable for quantitative analyses: (a) they are based on metaphase configurations, and the degree of contraction for various parts of the chromosome is not known, but can scarcely be assumed to be uniform; (b) they are based on studies of chromosomes of very small total length, and measurements cannot be made with high accuracy; (c) they are based on few loci and are therefore not susceptible to quantitative treatment. (3) MULLER has constructed a map of the X chromosome, in which the distances between loci are proportional to the frequencies with which mutations have been found in the regions between them ("mutation frequency" map) see MULLER and PAINTER 1932, pp. 321-8.

The basis for the construction of this mutation frequency map will be considered in some detail because of its great utility in reconstructing some of the events whose results we commonly observe as recombination of linked genes.

It is commonly assumed among geneticists that the genes hitherto found represent a random sample of all the genes in the chromosomes, and that, with the exception of regions demonstrated to be largely inert, genes are probably uniformly distributed throughout the lengths of the chromosomes. It is also generally assumed that the genes already reported are representative samples of the total gene population. To these may be added a third postulate, namely, that differences in mutation rate of various loci are not correlated with their positions in the chromosomes.

With these assumptions in mind, if all the genes which have been reported are arranged in their known order, the relative distance between any two genes may be expressed as the fraction of all the genes in that chromosome which lie between them.

The construction and use of the leptotene maps

The maps used in this paper differ in certain respects from the one drawn by MULLER (MULLER and PAINTER, loc. cit. 1932). For calculating the "mutation frequency" map, MULLER used the total number of mutations, and for the chromosome he used, namely the X, 570 mutations were available for inclusion in this map, for the most part lethals from X-ray experiments. In his map, however, rapidly mutating loci appear more frequently than do those with a lower rate of mutation, and multiple alleles are apparently counted each as a separate mutation. It seemed desirable to correct for these factors, and also to limit the cases selected to known point mutations. With these factors in mind, a census of all reported mutations on the X, II and III of *Drosophila melanogaster* was prepared. All cases which were suspected or known to be deficiencies or other than point mutations were eliminated. Multiple alleles and rapidly mutating genes were each counted only once. This method gives the closest approximation to a random sample at present available. As used in this paper, there were available 97 loci for the X chromosome, 88 for the II chromosome, and 81 for the III chromosome. The map of the X given below is in general agreement with the mutation frequency map of MULLER, although independently and somewhat differently derived. When larger populations of mutations are used in each chromosome (populations which have not been selected as probable point mutations), and maps drawn from these, only minor differences from the map here given are observed. In addition samples based on other criteria, for example lethals, Minutes, etc., also give essentially the same map. The samples used in this paper may therefore be regarded as representative and trustworthy. (For further discussion on this point see MULLER and PAINTER 1932.)

These maps are interpreted as giving the spatial distribution of the genes in the chromosomes relative to each other more accurately than at present available from any other source (for comparison with salivary maps, see below). It is not known whether these distances are uniform at all stages in the chromosome and life cycle. The stage of primary interest to the geneticist is that immediately preceding the occurrence of crossing over, and it is to this stage that the interpretation of distance is referred. In the absence of direct measurements at that particular stage, the best criterion whereby one may judge as to whether the maps do reflect the spatial relations at the time of or just before crossing over occurs, is by

examining the cogency of the relations obtaining when the above interpretation is given.

The analyses in the following pages are considered adequate in justifying this interpretation, and accordingly, the maps will be referred to as *leptotene* maps in order to characterize them as simply as possible.

The leptotene maps shown in table 1 were prepared by arranging all reported genes in each chromosome in the order that their reported locations require, and converting the total number of loci in each chromosome to a value of 100 percent. For example, the position on the leptotene map of the gene cut is at 50 on the X chromosome, which means that 50 percent of all the genes reported for the X chromosome lie to the left of cut. Likewise, placing the locus of the gene Stubble at 58.3 in III means that 58.3 percent of all the genes reported in the III chromosome lie to the left of Stubble.

TABLE 1
Leptotene maps of the three major chromosomes.

X		II		III	
LOCUS	GENE	LOCUS	GENE	LOCUS	GENE
0.0	yellow	0.0	aristaless	0.0	roughoid
22.4	white	4.1	Star	5.2	Crimped
24.5	facet	5.1	abrupt	12.5	sepia
29.6	echinus	11.2	dumpy	14.6	hairy
31.6	ruby	16.3	Streak	17.7	rose
37.8	crossveinless	18.4	Bristled	21.9	Minute-h
50.0	cut	23.5	dachs	25.0	Dicheate
59.3	lozenge	25.5	Ski _{II}	29.2	thread
62.3	vermillion	30.6	Jammed	32.4	scarlet
66.3	miniature	40.8	black	42.7	Deformed
75.5	sable	50.0	purple	44.8	pink
77.5	garnet	51.0	Bristle	50.0	maroon
88.7	rudimentary	60.1	cinnabar	51.1	curled
90.8	forked	63.3	vestigial	58.3	Stubble
92.8	Bar	67.3	Lobe	60.5	spineless
98.0	fused	73.5	curved	65.7	stripe
100.0	carnation	77.6	lance	74.0	Delta
		81.7	humpy	76.1	Hairless
		82.7	arc	77.2	ebony
		83.7	plexus	80.3	cardinal
		85.8	brown	89.6	rough
		91.9	speck	92.7	Pointed
		100.0	balloon	95.8	claret
				96.8	Minute
				100.0	Minute-g

It will be noticed that the leptotene map of the X chromosome ends with carnation at 100.0. This was done despite the fact that several genes are known to the right of carnation, because most of the chromosome be-

FIGURE 1.—Point by point comparison of genetic and leptotene maps of the three major chromosomes. Genetic maps here, and throughout this paper have been converted to 100 per cent. In II and III, where the spindle fibre insertion point is approximately median, the symmetry is about a corresponding point. In the X, with terminal insertion, the relation of the two maps is comparable to that of one arm of an autosome. The exceptional regions, y-w in the X, and a-ba in II are discussed in the text. The fourth pair of maps are drawn for II, with half the loci between arc and balloon excluded from the leptotene map, as indicated in the text.

yond carnation is inert genetically (MULLER and PAINTER 1932, DOBZHANSKY 1933). For purposes of discussion, it is more convenient to have the chromosome end at carnation because information available from several sources makes it probable that the inert region differs from the active part of the chromosome in its crossing over relations. Actually, however, if the leptotene map is drawn so as to include all genes up to bobbed, the gene at the extreme right end of the X chromosome, the calculations to which the leptotene map is put will not be materially affected. (On my map the locus of bobbed is 107.1).

Figure 1 shows a point by point comparison between the genetic (recombination) and leptotene maps of the three major chromosomes. Several things are apparent from a study of this figure. There is a noticeable regularity in the variation of the leptotene from the genetic maps as the distance from the spindle fibre changes. Chromosome III, for example, shows a symmetry almost exactly about the locus of pink which is the position for the spindle fibre reported by other investigators. Similarly in chromosome II, the symmetry is about a point between purple and Bristle, which also corresponds to the spindle fibre insertion previously reported. The X chromosome is asymmetrical with respect to the genetic map and varies from the rightmost end, which is likewise the end generally accepted, if not proved, as the position of the spindle fibre insertion.

In the leptotene maps, two regions seem to be aberrant, the leftmost region of the X between yellow and white, and the rightmost region of the II beyond humpy. The yellow-white region genetically giving only 1.5 percent of recombination, has a leptotene map distance of 22.4 units. There is a similar crowding of genes to the right of arc. It was thought that the crowding between arc and speck might perhaps reflect the fact that special efforts were made to retain all genes discovered in that region because of the interest in the "pale" translocation, which involved that portion of the chromosome. In an attempt to test this hypothesis, half of the loci between arc and balloon were arbitrarily excluded, and the remaining loci in the II chromosome were used to construct a "corrected" leptotene map. This is also included in figure 1. However, the results of this correction are unsatisfactory because in the first place, the crowding at the right end is not much improved by this treatment, and secondly, the regions in the center of the chromosome are shifted out of place so that the apparent locus of the spindle fibre becomes black, which is unlikely, judging from other data. It is concluded, therefore, that the exceptional regions at the right end of the II chromosome and at the left end of the X chromosome reflect some special conditions in these regions.

This test of the right end of II also indicates that whatever selection has been exercised in the reporting of new loci has not been such as to materi-

ally lessen the statistically representative character of the total loci available.

Changes in details of the leptotene maps will become necessary as further loci become available and are incorporated in them. Experience with these maps for the past year or more has shown that an increase of 10 percent in the number of loci used involves only small corrections, usually less than 1 percent. The error of any locus by this method is probably less than 3 percent.

To facilitate discussion, details of the computation in the rest of the paper have usually been omitted. A sample work sheet is included in the Appendix and the method of computation for the various tables and figures is briefly indicated.

Comparison of results obtained by using leptotene and genetic maps

When curves are drawn, figure 2, showing the change in fraction realized with distance in terms of the leptotene map for data on the completely marked X chromosome, it is found that interference progressively diminishes and then disappears about half the length of the chromosome. These results are sensibly uniform for all portions of the (active) chromosome, and they are practically unchanged when the distances are calculated in terms of ordinary genetic map units as given in figure 3. For the II chromosome, however, the results of these two types of plotting are not equivalent. In figure 4, the change in fraction realized with changing length of segments exchanged, in genetic units, is plotted. It will be seen that the results are variable. Especially in region 4, purple-cinnabar, which is in the vicinity of the spindle fibre, is the deviation most pronounced. When for the same data fraction realized is plotted against leptotene lengths exchanged, however, these variations practically disappear. In figure 5 regions 3, 4, 5, 6 and 7 are all on essentially one curve. *In this case the spindle fibre region falls completely in line with the results in the other regions.* It will be seen in this figure that the curves for regions 1 and 2 do not coincide with the curves for the other regions. It is considered that this remaining variation is due to the fact that there are undetected doubles within region 2 (dumpy-black) for which no corrections in the data have been made, and therefore regions to the left of it will have their results modified. This conclusion is supported by comparison of the curves for region 7, which, in figure 4, coincides with region 2, and falls on the average curve in the leptotene plot. It is to be expected that similar analyses of more comprehensive data in the same chromosome would yield still more uniform results. The similarity of the leptotene and genetic plots of the X is consistent with this finding for the II because of the terminal position of the spindle fibre in the X and the presence of a large amount of inert chromatin between it and carnation, which is the rightmost gene used.

FIGURE 2.—Change in interference with progressive increase in the size of segment exchanged for the X chromosome, in units of the leptotene map. Separate curves for each region fit the average curve. Compare with figure 6. Data of L. V. MORGAN unpublished, 3,570 flies.

FIGURE 3.—Same as figure 2 except that distances (abscissa) are measured in ordinary genetic units. The curve is similar to that of figure 2.

FIGURE 4.—Correlation between size of exchanged segment, in genetic units, and interference relations for the II chromosome. The curve for each region is the average of 28 points. The curves differ among themselves, especially region 4. Data of M. A. GRAUBARD 1932, 19,058 flies.

FIGURE 5.—Same as figure 4 except that segment length is measured in leptotene units. The curves correspond, except for regions 1 and 2, which are discussed in the text.

It is necessary to emphasize the necessity of using data on completely marked chromosomes in making analyses which will directly reflect the cytological events. The maximum amount of undetected doubles should not exceed 0.5 percent in any region and should be less than 1 percent for the entire chromosome (SCHWEITZER 1934, and unpublished calculations). This can be illustrated by a consideration of the data of BRIDGES and OLBRYCHT using alternated X-ple ($\frac{y \text{ } cv \text{ } v \text{ } f}{ec \text{ } ct \text{ } g}$). In plotting these data similar to figure 2, fraction realized against leptotene length, figure 6, it appears that the cut-vermilion region is aberrant in that it does not show the same rise in fraction realized as is shown by the other regions. When it is realized, however, that the data do not go beyond the locus of forked, it becomes clear that it is impossible for the cut-vermilion region which is in the middle of the chromosome to give interference relations comparable to the other regions because unless the chromosome is marked to the right-most end, there will be no region that will be independent of the cut-vermilion region in the experiment, and the fraction realized will consequently never rise to one. When the data of L. V. MORGAN on the more completely marked chromosome ($\frac{y^2 \text{ } cv \text{ } v \text{ } f}{y \text{ } ec \text{ } ct^6 \text{ } s \text{ } car}$) mentioned above are plotted however (figure 2), this anomalous result disappears. It is seen therefore that the use of the leptotene map has simplified the analysis by

FIGURE 6.—Same plot as figure 2 from data of BRIDGES and OLBRYCHT, 20,786 flies. In this case, region 4 (cut-vermilion) does not fit the average curve for the other regions. As explained in the text, this is due to the fact that the region to the right of forked was not marked in the experiment.

eliminating the spindle fibre as an influence in interference relations. Interference relations between regions are determined *only* by the physical distances between them.

Another practical demonstration of the greater effectiveness of the leptotene map as the basis for analyzing crossing over relations comes from the study of the effect of temperature on size of internode or length of section exchanged in a double crossover.

The recovery of gametes having such combinations of genes as make it possible to judge that two regions of interchange were involved in a single chromatid, makes it possible to estimate the internodal length. Referring again to the previous example, a 1, 4 double crossover would have a leptotene length equal to $1/2 (1+4) + 2 + 3$. This measure is obtained by plotting the frequency of double crossovers against the length of the internodal segment, and may be made for all regions of the chromosome. When such a plot is made for the normal II chromosome, at various temperatures between 14° and 30°, figure 7, the results obtained are sensibly uniform at all temperatures. When the plot is made using recombination percent as the abscissa, however, no general correlation can be made for the results at different temperatures, as can be seen in figure 8. Here again is seen the tremendously simplifying relationship obtained by using the leptotene plot. The temperature effect will be referred to again in the following pages.

These practical advantages would alone justify the use of the leptotene maps as the basis for analyzing the crossing over data and accepting them as representative of the spatial distribution at the time of crossing over. There is however in addition a theoretical advantage to be derived from the use of these maps. The recombination data as summarized in the genetic maps give the results of the operation of the forces involved in synapsis, exchange and disjunction, taken together. And it is impossible to analyze the components possibly contributed by each of these three forces to the end results, namely, recombination of linked genes. Since the leptotene maps are taken to give the location of the various genes in space in the chromosomes, they make possible a study of the mechanism of exchange by itself, because with their use, a study of this one variable alone may be made.

ANALYSIS OF CROSSING OVER IN THE NORMAL CHROMOSOMES

It was consistently found during the course of the calculations that only data involving good and equally viable contrary classes and involving fairly large numbers covering chromosomes completely marked, gave clear-cut results when analyzed, as here indicated. Consequently, it was not possible to check up each conclusion from one set of data with all the other extant data that might otherwise be relevant. The general principle

FIGURE 7.—The frequency of double crossover classes of different leptotene lengths, at four temperatures for the II chromosome. The average curve clearly fits the data at all temperatures. The large dotted circles are the average for all temperatures, each corrected for its own interference value. It is apparent that there are no important differences between the two curves. Data of M. A. GRAUBARD.

FIGURE 8.—Similar to figure 7 except that the abscissa is in genetic units. In contrast to figure 7, no single curve can be drawn. For example, at lengths 40 and 50, the different temperatures have widely different frequencies of doubles, and the curves for several temperatures are going in contrary directions.

has been followed, however, in placing most credence on the results obtained from what seemed to be the best data, judging by the criteria indicated above. However, every case of important or substantial disagreement between the results, obtained from analyzing different sets of data, has been indicated.

The X chromosome

For the study of crossing over in the X chromosome, the following data were used: X-ple, BRIDGES and OLBRYCHT 1926 (20,786 flies) and the unpublished data of L. V. MORGAN referred to above (3,570 flies). In

FIGURE 9.—Frequency of double crossovers for different leptotene lengths of the X. This curve appears modal at about 30 units. Data of L. V. MORGAN.

FIGURE 10.—The same plot as figure 9 with a correction factor applied to the frequency of doubles in each class, to correct for the inequalities in length of chromosome between the loci used in the experiment. There is no modality in this figure.

addition, some calculations were made on the control data of L. V. MORGAN, 1933 (*ec-bb*, 2,623 flies).

Interference progressively disappears as the distance from the region of first interchange increases, becoming negligible beyond about half the total length of the X. This has already been demonstrated (SCHWEITZER loc. cit. tables 1, 2 and 5) and in figures 2, 3 and 6 above.

When double crossovers are plotted against leptotene length, as in figure 9, the results might be interpreted as demonstrating the presence of a modal length for the internodal segment. That this mode is spurious rather than real is shown when the same plot is made except that each region is corrected for the frequency of expected crossing over within the regions involved. This correction is necessary because the loci used in the experiment are not equidistant from each other. For example, scute-echinus is 7.5 units, cut-vermilion is 13.0 units. With the same frequency of occurrence of crossing over, there should be 1.7 times as many crossovers in the cut-vermilion region as occur in the scute-echinus region. The correction is made by multiplying each frequency by an appropriate factor derived from the expected amount of crossing over involved in each of the two regions due to this factor. Figure 10, which has been derived in this way, shows that the frequency of double crossovers increases as interference lessens and remains constant for all distances not involving interference. If now of the corrected doubles, those involving interference are

FIGURE 11.—Proportion of doubles that involves interference in the X. In the lower curve the doubles are corrected as in figure 10, and summated in order of length, and the doubles involving interference are also summated separately. In the upper curve, the data is also corrected for interference before summated. Sixty-two per cent of all doubles that occur involve interference. Eighty-three per cent of those that would occur involve interference. Data of L. V. MORGAN.

separated from the rest and summated for increasing distance and compared with the total number of corrected doubles, as in the lower curve of figure 11, the indication is that about 2/3 (62 percent) of all nodes that form (measured genetically as double crossovers) involve interference. The upper curve in the same figure, which has also been corrected for inter-

ference at every point, indicates that about 4/5 (82 percent) of all nodes that attempt to form (measured by interference) do in fact interfere with each other.

That the results are uniform for all regions may be shown by comparing the frequency of double crossovers for each region separately. These have not been reproduced but all give a fit to the average curve.

Another measure of the effectiveness of interference in different parts of the chromosome may be made by studying the size of independent regions in various parts of the chromosome. This is obtained by interpolating for each region of the map the leptotene length that must be included before interference just disappears. Table 2, which includes the values from all available data, indicates that this is sensibly constant throughout the chromosome length.

TABLE 2

Length of intervening segment, leptotene units, from a region where a crossover has occurred to the region where another occurs without interference due to the first.

REGION	LOCUS OF MIDPOINT	LENGTH OF SEGMENT (INTERPOLATED)			AVERAGE
		B. & O. 1926	L.V.M. 1933	L.V.M. UNPUB.	
<i>y-ec</i>	15			70	70
<i>sc-ec</i>	15	65			
<i>ec-cv</i>	34	50	50	55	50
<i>cv-ct</i>	44	50	40	60	50
<i>ct-v</i>	56	60	60	50	55
<i>v-s</i>	69			50	50
<i>v-g</i>	70	60	45		
<i>s-f</i>	84			55	50
<i>g-f</i>	83	50	40		
<i>f-car</i>	95			50	50
<i>f-bb</i>	99		50		

A few words should be said on the meaning of the term "loop length." This term, as commonly defined and used, refers to the internodal length. It has been used to refer to the modal length of internodal segment. But in view of the absence of modal loop lengths, it is necessary to revise the terminology. In this paper, the term "loop length" is not used; the more general term "internodal length" refers to segments of interchange involved in a double crossover (BRIDGES 1915, 1929).

The II chromosome

For this chromosome, the data of GRAUBARD 1932 and of REDFIELD 1932 have been analyzed. In REDFIELD's data, the contrary classes are unequal in many cases, and this, in addition to the fairly small numbers involved,

sometimes led to irregularities in the results when plotted. In all cases, however, where comparable figures could be drawn, the results were in agreement with those obtained from GRAUBARD'S data.

The data on fraction realized have already been discussed in connection with the leptotene maps (figures 4 and 5), and also in connection with the use of fraction realized as a measure of interference (see SCHWEITZER *loc. cit.* tables 3 and 6).

The plotting of internodal lengths in figure 7 seems to show the existence of a mode. This mode, like the one in the X chromosome (figure 9) is

FIGURE 12.—Frequency of double crossovers of different sizes corrected for non-equidistance of the intervals between the marked loci for the II chromosome. This figure is inconsistent with the interpretation of modal length of segments in double crossing over. Data of M. A. GRAUBARD.

FIGURE 13.—Interference and non-interference double crossovers in the II chromosome. Twenty-five per cent of all doubles involve interference. An additional 25 per cent fail to appear due to interference. Data of M. A. GRAUBARD.

spurious, however, for when the data are corrected in the manner indicated for the X chromosome, this supposed modal length also disappears. Figure 12 shows clearly that for the II chromosome there is no modal distance between the two breaks in double crossing over. Separate plots for each region, not included here, show that in this chromosome, as in the

X, the internodal lengths are similar in distribution for all regions of the chromosome.

It has already been pointed out that the two arms behave like two independent chromosomes. It might perhaps therefore be expected that analyses of data in each arm taken separately would yield different results, but this does not turn out to be the case. The internodal lengths within a single arm have a modal value only if the data are not corrected for the expectancy of crossing over in the various regions (curves not given).

FIGURE 14.—Size of regions giving no interference in double crossing over in the II chromosome. The curve may be interpreted as symmetrical about the spindle fibre. Data of H. REDFIELD (1932) and M. A. GRAUBARD (1932).

FIGURE 15.—Effect of temperature on fraction realized in the II chromosome. There is no temperature effect on interference. Data of M. A. GRAUBARD.

It is possible also in this chromosome to estimate the frequency of interference loops. It turns out in this case, figure 13, that about 25 percent of all recovered internodal segments show interference, and an equal number fail to appear due to the effect of interference.

It is possible also to study directly the effect of interference upon internodal length. Correction of graphs such as figure 7 for interference

shows, large dotted circles in figure 7, that interference does not affect the frequency distribution of internodes.

For the II chromosome, the size of independent regions is not constant throughout the chromosome, as was the case in the X chromosome. Figure 14 indicates that the size of independent regions varies symmetrically about the spindle fibre.

The III chromosome

For this chromosome, there was no single set of good data available for study. The curves of the analyses represent, in part, studies from data of BRIDGES (1929), in which the region to the right of sooty was not marked, several sets of data by DOBZHANSKY (1930, 1932) which were published as controls for various translocation experiments, and the data on normal chromosomes published as controls by REDFIELD (1930) and BEADLE (1932) summarized together. Only in the case of BRIDGES, were the contrary classes fairly equal. The other data had to be used, however, since in BRIDGES' experiment a large portion of the right limb of the chromosome was unmarked. Since the curves for this chromosome are all similar to the preceding ones, none have been reproduced.

The plot of fraction realized for all three sets of data is in general similar to that of GRAUBARD on the II chromosome. A characteristic of all three curves is that the fraction realized regularly rises to values greater than 1.0. This may be interpreted as being due: (a) to the larger size of the III chromosome (it is reported to be $1/3$ larger than the II cytologically) and (b) to the presence in it of a larger average number of chiasmata than occur in the II chromosome (MATHER 1933, having shown from the analysis of diploid data that the average number of crossovers is 1.94 in II and 2.4 in III).

It might be expected from this that a modal length would be found in this chromosome. A plot of corrected internodal length similar to those for X and II from REDFIELD's and BEADLE's and DOBZHANSKY's data shows the absence of modal values. BRIDGES' data give an apparent modality but his data is unsuitable for this type of calculation because of the presence of a long terminal unmarked region, as was shown in the discussion of the X-ple data (figure 6 above). As in the case of the II chromosome, when non-interference internodes are plotted separately, there is no indication of modality. More detailed analyses of each single region in these data make the conclusion inescapable that there is no modal length because each separate region gives an approximate fit to the curve for the data as a whole. Such figures also show that there are no regional differences throughout the length of the III chromosome.

Since the best data for this chromosome do not cover the entire map,

no analysis of the size of independent regions in all parts of this chromosome can be made.

VARIATIONS IN CROSSING OVER

Temperature

The effect of temperature on crossing over has been studied by several observers, PLOUGH (1917, 1921), STERN (1926) and GRAUBARD (1932). Only in GRAUBARD's data, are available variations in crossing over in chromosomes completely marked.

Temperature changes above and below 25° increase the crossover values in the II chromosome near the spindle fibre, but not in the more distal regions. Because of the regional differences, changes in internodal length and in interference relations may be profitably studied for this data, to determine the influence of crossing over on internodal length, interference, etc. Some of the results have already been indicated.

As far as has been determined, temperature has no effect whatever on

TABLE 3

Fraction realized and proportion of double crossovers for all temperatures in II.

CLASSES	3A. FRACTION REALIZED					3B. PROPORTION OF DOUBLE CROSSEOVERS				
	25°	14°	16.5°	30°	AVERAGE	25°	14°	16.5°	30°	AVERAGE
1,2	0.2	0.3	0.4	0.2	0.28	2.8	2.8	3.4	4.4	3.4
1,3	0.5	0.7	0.6	0.8	0.65	1.8	3.3	2.7	2.9	2.7
1,4	1.0	1.4	0.9	1.2	1.13	0.9	1.1	1.0	1.9	1.2
1,5	1.0	1.0	0.9	1.0	0.98	4.1	3.1	3.5	3.1	3.5
1,6	0.9	1.5	1.1	1.0	1.13	2.0	1.8	1.8	1.5	1.8
1,7	1.1	1.2	0.9	1.1	1.08	10.6	7.0	6.7	8.2	8.1
2,3	0.2	0.4	0.5	0.5	0.40	1.6	7.0	4.2	4.3	4.3
2,4	0.8	1.0	1.1	0.5	0.85	2.0	3.5	3.2	4.3	4.3
2,5	1.2	1.0	1.0	0.9	1.03	13.4	14.0	12.3	10.3	12.5
2,6	0.9	0.9	1.1	1.0	0.98	4.9	4.7	5.7	3.8	4.8
2,7	1.1	0.9	1.0	1.1	1.03	31.7	17.8	27.2	22.4	24.8
3,4	0.7	0.2	1.8	0.4	0.78	0.7	0.5	0.8	1.6	0.9
3,5	1.1	0.8	0.8	1.1	0.95	2.8	5.2	4.1	4.3	4.1
3,6	1.0	0.9	1.0	0.9	0.95	1.2	2.1	1.7	1.6	1.7
3,7	1.0	1.0	0.9	0.8	0.93	6.9	11.3	8.6	6.4	8.5
4,5	0.4	0.3	0.4	0.5	0.40	0.5	0.5	0.6	1.1	0.7
4,6	0.5	0.7	0.2	1.0	0.60	0.3	0.5	0.4	0.9	0.5
4,7	0.7	0.8	0.7	0.8	0.75	1.9	4.8	2.3	4.4	3.4
5,6	0.4	0.6	0.8	0.6	0.60	1.0	0.1	0.3	0.9	0.6
5,7	0.6	0.8	0.6	0.7	0.68	6.7	10.7	7.9	8.2	8.4
6,7	0.4	0.1	0.3	0.6	0.35	2.0	1.4	1.8	3.3	2.1

the general relationships. In table 3a, the average value of fraction realized for each class at all temperatures, is indistinguishable from the results obtained at 25°. Table 3a gives the fraction realized of each internodal segment for each temperature. This shows that the interference relations are unchanged. Figure 15, which is a detailed comparison at each temperature of region 1, and is representative of conditions throughout the chromosome, shows further that there is no constant effect associated with any particular temperature, indicating that the mechanism is unaffected. Table 3b shows that the proportion of internodes is closely comparable for all temperatures, and that in this case also no systematic differences occur. There is likewise no modal length of internode at any temperature.

Age

Variations in crossing over in relation to the age of female was first reported by BRIDGES in 1915 on the II chromosome. Since that time, studies of the effect on each of the chromosomes have been made by PLOUGH (1917, 1921), STERN (1926), BRIDGES (1927, 1929), BERGNER (1928). The only data on the age effect which is sufficiently comprehensive to be useful for analyzing the results on the general mechanism of crossing over is that of BRIDGES on the III chromosome. In this experiment, seven loci were simultaneously followed covering all of the left arm and $\frac{1}{2}$ of the right arm, up to sooty (*e*^{*}). Females were transferred to fresh food every two days, so that it is possible to compare the results at close intervals. BRIDGES found that crossing over was decreased from an original maximum in the first three days to a minimum at about the ninth or eleventh day, an average of 36 percent for the entire chromosome, with relatively unimportant changes thereafter. He also reported changes in coincidence relations with age. As in the case of temperature, variations in crossing over with age are not uniform for all parts of the chromosome.

A study of the interference changes during the early days of egg-laying, when the crossing over changes are most pronounced, reveals no consistent changes with age. Computations were made for many combinations of egg-laying periods. Three kinds of calculations are given in table 4 for each of ten egg-laying periods, which would be expected to show the widest differences among themselves.

In table 4a, the values of fraction realized for each of these ten egg-laying periods are quite comparable to each other and to the results of the normal period (0–11 days), which has been discussed in connection with the analysis of the III chromosome.

The corrected frequency of double crossovers for these egg-laying periods given in table 4b are quite comparable in all respects to similar calculations on the standard III chromosome. The apparent modality is considered spurious for the reasons previously mentioned.

TABLE 4
Fraction realized, corrected doubles and length of independent regions for various egg-laying periods in III.

	CLASSES	EGG-LAYING PERIOD									
		0-39	0-31	0-21	0-11	0-5	5-11	11-17	11-21	21-31	31-39
4A. FRACTION REALIZED	1, 2	0.2	0.2	0.2	0.2	0.3	0.6	0.7	0.3	0.2	*
	1, 3	0.1	0.8	0.8	1.3	0.9	0.9	1.0	0.7	0.6	0.0
	1, 4	1.1	1.4	1.4	1.7	1.9	1.0	0.9	0.7	0.5	*
	1, 5	1.3	1.3	1.3	1.5	1.8	1.2	1.1	1.0	0.2	*
	1, 6	1.5	1.5	1.5	1.3	1.5	1.2	1.7	1.7	1.2	2.4
	2, 3	0.1	0.2	0.2	0.3	0.2	0.3	0.0	0.0	0.0	0.0
	2, 4	0.7	0.5	0.5	0.7	0.6	0.6	0.3	*	1.0	*
	2, 5	1.1	1.2	1.2	1.0	1.2	1.2	*	0.9	0.9	1.8
	2, 6	1.3	1.2	1.2	0.9	1.0	1.1	1.4	1.6	1.1	1.1
	3, 4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3, 5	1.6	1.5	1.3	1.1	0.3	3.0	*	*	*	*
	3, 6	0.7	0.7	0.9	0.7	0.3	1.4	0.0	0.9	0.0	0.0
	4, 5	1.5	1.5	1.7	1.7	1.1	2.8	*	0.8	*	0.0
	4, 6	1.0	1.0	0.9	1.1	1.3	0.3	*	*	1.1	1.6
	5, 6	1.6	0.4	0.3	0.3	0.3	0.4	0.3	2.5	0.6	0.0
4B. CORRECTED DOUBLES	1, 2	3.2	3.3	3.2	2.9	2.1	3.3	3.6	4.0	4.5	*
	1, 3	10.0	8.0	6.6	7.3	6.2	10.9	1.5	0.7	*	0
	1, 4	18.0	12.5	13.2	12.2	8.9	16.1	14.3	10.8	7.5	*
	1, 5	18.0	12.2	12.5	10.4	8.0	13.1	22.4	13.0	18.6	*
	1, 6	13.0	12.4	12.1	9.6	6.8	13.9	20.0	15.4	17.0	1.6
	2, 3	4.7	4.0	5.0	6.2	1.6	11.2	0	0	0	12.9
	2, 4	14.3	7.5	7.0	7.0	4.1	13.0	*	*	2.4	*
	2, 5	20.4	13.0	13.0	11.8	8.0	18.0	15.2	16.3	18.0	13.5
	2, 6	14.7	12.9	12.0	9.3	5.5		18.1	17.1	20.0	6.4
	3, 4	1.4	1.0	5.0	5.7	0.0		0	0	0	*
	3, 5	13.0	14.0	18.6	12.6	*		*	*	*	36.2
	3, 6	4.3	10.0	13.0	8.1	*		0	0.8	0	0
	4, 5	17.2	20.0	22.0	14.5	5.7		*	13.0	*	20.1
	4, 6	8.9	11.5	11.0	10.0	7.4		*	*	1.5	1.7
	5, 6	3.9	4.9	5.0	4.1	3.2		6.7	5.2	7.5	4.3
4C. LENGTH OF INDEPENDENT REGION	Region										
	1	4	4	4	3	4	4	3	5	6	*
	2	5	5	5	5	5	5	6	6	4	5
	3	5	5	5	5	7	6	*	*	*	*
	4	5	5	5	5	5	5	*	*	6	6
	5	4	4	4	4	4	4	*	*	*	*
	6	5	4	2	4	4	3	2	5	4	4

* Numbers too few to be significant.

Calculation of the size of independent regions for these different egg-laying periods reveals no apparent change with age (table 4c).

DISCUSSION

The role of the spindle fibre region in crossing over has been clarified. The spindle fibre separates the autosomes of *Drosophila* into two units with independent genetic behavior. This conclusion is consistent with the observations on the salivary gland recently reported by PAINTER (1933, 1934), in which the arms of the autosomes appear physically independent of each other. It may be compared with the condition found in other insects (summarized in WILSON 1925), where independent chromosomes sometimes become attached during the meiotic stages. In this case, the attachment is persistent in germinal tissues, and the separation, when it occurs, takes place late in embryogeny. The spindle fibre has no specific effect on the process of exchange. Regions in its immediate neighborhood behave in all respects as do regions further removed.

If exchange usually starts at some definite point, it might be expected that the cytological lengths of chromatin showing interference would be changed when crossing over is changed. The absence of any effect of age and temperature on interference, when both are known to change the recombination values, and have a differential effect in different regions of the chromosomes, would seem to indicate that there is no definite region where crossing over first occurs and from which the frequency of crossing over in other regions is influenced. Crossing over after pairing occurs independently (and perhaps simultaneously) throughout the length of the chromosome.

Many of the problems associated with interference have already been discussed. Interference is revealed as a local phenomenon associated with regional events but with no central determining mechanism. Interference therefore need not necessarily be interpreted as a mechanical factor affecting the chance of an exchange at any locus; it may be a reflection of the mathematical generalization that in a chromosome having only a limited number of crossovers, relatively few of them will occur close together. This interpretation, if supported, would negate most of the current hypotheses regarding the mechanism of interference as due to twisting of the chromosome, breakage, etc. The published data on inversions (GRAUBARD 1932, 1934) support this conclusion, and preliminary calculations of interference and the cytological properties of inverted and translocated chromosomes give results wholly consistent with this interpretation. But at present, the evidence is insufficient to decide between these alternatives. In triploids, the published data make it probable that change of partners occurs with sufficient frequency so that interference is very much

decreased, and it is to be expected therefore that calculations from the data on crossing over in triploids, when corrected as indicated in this paper, will give recombination percentages for all regions equivalent to those appearing in the leptotene maps.

The phenomenon of recombination, as already mentioned, reflects the operation of forces of pairing, exchange and disjunction. Similarly, theories of crossing over require analyses and hypotheses regarding the interaction of these three kinds of forces. The material presented in this paper is concerned primarily with the forces of exchange, including the location of the genes at the time of exchange, the formation of overlaps and the internodes between succeeding overlaps, and in addition, the problem of interference. Pending the conclusion of analyses involving situations in which the chromosomal configuration has been changed, translocations and inversions, where the forces of attraction (pairing) are affected, and of the data on non-disjunction where the forces of disjunction are interfered with, it is premature to attempt to pass judgment on current hypotheses regarding the mechanism of crossing over. All theories of crossing over now current are inadequate in their analysis of interference.

If the recently described salivary chromosomes represent the normal distribution of genes in all tissues, it will be possible to make analyses equivalent to those here presented from salivary maps as soon as enough loci are identified. At present, the most detailed salivary map is of the X. It is consistent with the leptotene map and yields similar results on analysis. This cannot be taken as verification of the leptotene maps, however, since the leptotene map of the X is not very different from the genetic map. A more critical comparison will be possible when detailed salivary maps of II and III appear.

From the manner of their construction, the leptotene maps exclude the inert regions from consideration. This is inevitable because our information about crossing over is limited to those portions of the chromosomes that have *active* chromatin (by which is commonly meant regions that (a) contain genes which mutate with measureable frequency, and (b) the loss or addition of any considerable portion of which has a marked influence on the viability, somatic characteristics, etc. of the tissue concerned). The localization of large amounts of inert chromatin in the vicinity of the spindle fibre of the three major chromosomes may afford a clue to the effect of age and temperature, whose crossing over effects are also largely localized near the spindle fibre.

A theoretical difficulty common to all analyses of *Drosophila* data on crossing over, including this one, is that they are based on the recovery of single chromatids while the reference is intended to the tetrad. Until the role of the forces in the tetrad, especially the interrelation between

exchange and disjunction is clarified, this weakness will continue to be important. The simplicity of the relations and the mutual consistency of diverse types of data on all chromosomes as presented in this paper, renders it probable, however, that this and other uncontrolled factors are of secondary importance.

SUMMARY

1. The data and concepts of coincidence and interference are analyzed, and a new method of calculating interference relations is suggested.

2. Interference relations are uniform for the X chromosome, which has a terminal spindle fibre insertion region.

3. Interference relations for the II chromosome and the III chromosome are likewise uniform. These have a median spindle fibre insertion. A discussion of the role of the spindle fibre and of interference is included.

4. New maps of the chromosomes of *Drosophila* are constructed. These "leptotene" maps are interpreted as giving the distribution of the various loci at the time of crossing over.

5. There is no modal length of internodal segment in X, II or III chromosomes. For regions not involving interference, internodes of all sizes are equally likely to occur.

6. Two-thirds of the crossover chromatids in the X involve interference.

7. Twenty-five percent of the crossover chromatids in the II chromosome involve interference.

8. Interference does not affect the cytological length of internodal segment.

9. Crossing over does not begin at any particular place on the chromosome.

10. Temperature does not influence the values of interference, the frequency of internodal length, or the size of independent regions.

11. Age changes in the female do not affect the values of interference, the frequency of internodal length, or the size of independent regions.

12. It is concluded that temperature and age changes have no effect on the general mechanism of crossing over, but that each has only specific and regional effects on the frequency of occurrence of crossing over.

13. The absence of any general effect of age and temperature justifies the use of a ten-day egg-laying period and 25° as standardized conditions for the acquisition of data for the analysis of the mechanism of crossing over.

APPENDIX

Computations

The tables and graphs have been made directly from work sheets, of which one is given in the accompanying table.

L.V.M. unpub.

X-chromosome, 25°

y-ec-cv-cl-v-s-f-car
1 2 3 4 5 6 7

#6A-2

#=3570 flies

N=total of (d+t)=410

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15				
CLASS	SIZE	GENETIC STANDARD		LEPTOTENE LENGTH	COINCIDENCE PERCENT	FRACTION REALIZED	$a \times b \times 10^{-2}$	$d+t$	$\Delta d+t$		DOUBLES	COLUMN 11		COLUMN 12		DOUBLES		
		IN LENGTH	IN PERCENT						$d+t$	N		$a \times b$	IN PERCENT	FR. REAL.	SUMMATED		SUMMATED	FR. REAL.
1,2	6	8.2	11.0	18.9	0.1	0.0+	0.65	3	0.7	9.8	1.1	10.4	7.7	32.1	6.9			
1,3	11	17.0	22.6	29.1	0.1	0.0+	0.56	2	0.5	34.8	0.8	8.6	27.9	58.1	2.5			
1,4	15	29.0	38.0	41.3	0.9	0.8	1.07	34	8.3	63.6	7.4	4.8	54.5	75.7	5.2			
1,5	18	42.0	56.4	53.9	0.6	0.6+	0.69	14	3.4	83.6	4.8	3.8	75.7	87.1	4.1			
1,6	20	53.6	75.5	68.2	0.9	0.9+	0.90	30	7.3	96.0	7.9	4.3	91.2	94.7	3.6			
1,7	21	63.6	91.1	80.4	1.0	0.8+	0.46	17	4.2	100.2	8.7	5.3	99.9	100.0	2.1			
2,3	1	8.9	11.6	10.2	0.0	0.1	0.78	2	0.5	0.5	0.6	2.8	0.6	2.8	2.5			
2,4	7	20.9	27.0	22.4	0.5	0.6	1.50	29	7.1	16.9	4.5	3.8	12.2	35.9	5.9			
2,5	12	33.9	45.4	35.0	0.8	0.8	0.96	28	6.8	41.6	6.9	4.2	34.8	62.3	4.1			
2,6	16	45.5	64.5	49.3	1.2	1.5	1.25	52	12.7	76.3	9.7	3.3	64.2	79.0	6.2			
2,7	19	55.5	80.1	61.5	0.9	1.1	0.65	21	5.1	88.7	7.6	3.3	83.3	90.4	2.5			
3,4	2	12.0	15.4	12.2	0.2	0.1	1.30	8	2.0	2.5	1.5	7.1	2.1	9.9	9.8			
3,5	8	25.0	33.8	24.8	0.6	0.5	0.83	19	4.7	21.6	5.4	5.2	17.6	41.1	4.6			
3,6	13	36.6	52.9	39.1	0.6	0.5	1.08	23	5.6	47.2	5.0	4.8	39.8	67.1	5.5			
3,7	17	46.6	78.5	51.3	0.8	0.8+	0.56	16	3.9	80.2	6.7	4.3	70.9	83.3	2.1			
4,5	4	13.0	18.4	12.7	0.3	0.3	1.60	20	4.9	8.1	3.0	4.7	5.9	18.4	8.0			
4,6	10	24.6	37.5	27.0	0.5	0.5	2.09	36	8.8	34.3	4.0	3.7	27.1	49.5	8.6			
4,7	14	34.6	53.1	39.2	0.9	0.9	1.07	33	8.3	55.3	7.3	3.8	47.1	70.9	4.5			
5,6	5	11.7	19.1	14.3	0.1?	0.1	1.33	4	1.0	9.1	0.7	3.3	6.6	21.7	4.9			
5,7	9	21.7	34.7	26.5	0.7	0.6	0.69	16	3.9	25.5	5.5	4.7	23.1	45.8	3.2			
6,7	3	10.0	15.6	12.3	0.1	0.1	0.90	3	0.7	3.2	0.8	3.8	2.9	13.7	3.4			

In Column 1, the classes are arranged in order of size as measured in leptotene lengths. Class 2, 3 is the smallest; 3, 4 next larger, etc.

Column 2 gives the length of each class in genetic units as computed directly from the original data. The position of the breaks is taken to be midway between the loci followed in each case. For class 1, 4 the length is equal to $1/2 (1+4)+2+3$, which equals $1/2 (6.8+15.8)+9.5+8.2 = 29.0$. The total map is made equal to 100 units.

Column 3 gives the length of each class in standard genetic units from the latest published maps of BRIDGES (in T. H. MORGAN, *The Scientific Basis of Evolution*, 1932). Each map is converted to percent.

Column 4 gives the length in terms of the leptotene map as described in detail in the text.

Column 5 gives the coincidence as computed by WEINSTEIN's method, eliminating triples involving intermediate loci.

Column 6 gives fraction realized computed as described in the text.

Column 7 gives the product of the total genetic length of the first region, represented by a , and the length of the second region, b for the given experiment. For class 1,4 $a=1=6.8$, $b=4=15.8$, and $1 \times 4 = 1.07 \times 10^{-2}$.

Column 8 lists the total double crossover classes taken directly from the original data. For 1,4 they are 1,4-32; 1,4,6-2; total, 1,4=34.

Column 9 is obtained by dividing each number in column 8 by 410, which is the total number of double crossovers in column 8. For 1,4, $34/410 = 8.3 \times 10^{-2}$.

Column 10 is a summation of column 9 in order of size from column 1. For 6,7, it is equal to 2,3+3,4+6,7 or in numerical figures, $0.5+2.0+0.7 = 3.2$.

Column 11 is column 9 divided by column 7 and converted to 100 units.

Column 12 is column 11 divided by column 6 and converted to 100 units.

Column 13 is summation of column 11 in order of size.

Column 14 is summation of column 12 in order of size.

Column 15 is column 9 divided by column 6 and converted to percent.

* * * * *

The tables and figures referred to in the text were drawn up as follows:

Table 1. The locus of any point in this table is the fraction of all recorded genes in that chromosome that lie to the left of it.

In figure 1, the corresponding loci on the leptotene and genetic maps are connected. The total length of each map has been made equal to 100 units.

Figures 2, 5, 6 and 15 have as their co-ordinates columns 6 and 4. In table 6, column 2 is from table 1, and columns 3, 4 and 5 are interpolated from graphs like figure 2.

Figures 3 and 4 have as their co-ordinates columns 6 and 2.

Figures 7 and 9 have as their co-ordinates columns 9 and 4, except for the large dotted circles in figure 7, which have columns 15 and 4.

Figure 8 has as its co-ordinate columns 9 and 2.

Figures 10 and 12 have as their co-ordinates columns 11 and 4.

In figure 11, the upper curves, open circles, have as their co-ordinates columns 14 and 4. The smaller circles are on the curve from which regions having no interference have been omitted. The lower curves, solid circles, have as their co-ordinates columns 13 and 4. The smaller circles here also exclude non-interference regions.

In figure 13, crosses and solid center circles have as their co-ordinates columns 13 and 4, except that classes giving interference are separately summated and drawn. The open circles have as their co-ordinates columns 15 and 4. In this case, only the classes giving interference are drawn and they have been summated in the figure.

Figure 14 corresponds to column 6 of figure 6.

Tables 3a and 4a are reproductions of column 6.

Table 3b is column 9 reproduced for all temperatures.

Table 4b is column 11 reproduced.

Table 4c is taken from the column of classes at the point where column 6 reaches 1.0.

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