

TEMPERATURE EFFECT ON INTERFERENCE AND CROSSING OVER

MARC A. GRAUBARD¹
University College, London, England

Received April 4, 1933

A consideration of the data presented in an earlier paper (GRAUBARD 1932) brings out certain facts with regard to the mechanism of crossing over in chromosome II of *Drosophila melanogaster* which may have general significance. In the previous work experiments were performed to study crossing over and interference along the entire length of the normal chromosome II and of the same chromosome with a median inverted section of an estimated length of 25 units. The cross was arranged so as to have eight marked genes along the chromosome covering it from one end to the other. The genes employed were aristaless (3.0) dumpy (13.0) black (48.5) purple (54.5) cinnabar (57.5) vestigial (67.0) Lobe (72.0) and speck (107.0) (MORGAN, BRIDGES and STURTEVANT 1925).

If the data given in tables 8 and 9 of the previous paper are restated so as to show the relative proportions of crossover and non-crossover flies, we get the following:

TABLE 1
Normal chromosome.

CROSSOVERS	14°C	PERCENT	16.5°C	PERCENT	25°C	PERCENT	30°C	PERCENT
0	862	24.5	1417	24.7	1623	30.7	1218	27.0
1	1608	45.7	2629	45.8	2528	47.8	2008	44.5
2	876	24.9	1491	26.0	1043	19.7	1067	23.6
3	157	4.5	190	3.3	85	1.6	201	4.6
4	9	0.25	12	0.2	5	0.1	17	0.4
5	0						2	
6	0						1	
Total	3512		5739		5284		4514	

TABLE 2
Homozygous inversion-bearing chromosome.

CROSSOVERS	14°C	PERCENT	16.5°C	PERCENT	25°C	PERCENT	30°C	PERCENT
0	1094	30.8	1675	31.3	3221	34.2	1319	26.0
1	1686	47.5	2508	46.9	4389	46.7	2170	44.2
2	677	19.0	1053	19.7	1635	17.5	1180	24.1
3	87	2.3	101	1.9	146	1.5	212	4.3
4	4	0.1	5	0.1	7	0.07	14	0.3
5							4	0.1
Total	3548		5342		9398		4899	

¹ Fellow of NATIONAL RESEARCH COUNCIL.

These results indicate that taking the chromosome as a whole the number of non-crossovers is about 25 percent of the total at 30°C, and invariably higher at 25°C. The rest of the flies are distributed among the various types of single and multiple crossovers. In the same paper experiments were also performed on crossing over in only one arm of the second chromosome, a procedure made possible by introducing an inversion in the respective other arm and keeping it heterozygous. Such a condition allows crossing over to occur freely in only one arm. It must be borne in mind that the two arms are joined at the point of spindle fiber attachment to form a V-shaped chromosome. Restating these data (tables 7 and 6) as before, we get the following:

TABLE 3
Heterozygous left inversion. Crossing over takes place in right arm only.

	14.5°C	PERCENT	25°C	PERCENT	30°C	PERCENT
0	2042	55.6	601	53.1	1039	48.7
1	1518	41.5	495	43.8	942	44.2
2	96	2.7	34	3.0	145	6.8
3	1	0.1	1	0.1	4	0.2
Crossovers	1615	44.4	530	46.9	1091	51.3
Total	3657		1131		2130	

TABLE 4
Heterozygous right inversion. Crossing over takes place in left arm only.

	16°C	PERCENT	25°C	PERCENT	30°C	PERCENT
0	2178	54.2	2225	59.9	863	51.6
1	1757	43.7	1437	38.7	725	43.3
2	79	1.9	51	1.4	77	4.6
3					4	0.3
Crossovers	1836	45.8	1488	40.1	806	48.4
Total	4014		3713		1669	

In this case we see that non-crossovers are about twice as frequent as before, giving values of approximately 50 percent at 30°C, and higher figures for the other temperatures. It is therefore apparent that whenever only one arm of this V-shaped chromosome is concerned the chance for at least one crossover is approximately 1/2 at 30°C, but that when the entire chromosome is involved the chance for a crossover is 3/4. At 25°C the probability for getting a crossover in each arm is less than 1/2 and for the entire chromosome the chances are less than 3/4. It should be remembered that the data of DOBZHANSKY (1931) show that the point of spindle fiber attachment is barely to the right of purple and that the two arms are approximately of the same length.

Tables 1 and 2 seem to show that except at 30°C the percentage of non-crossovers is higher for the inversion-bearing chromosome than for the normal.

The data of tables 8 and 9 can be further restated so as to show whether the probability for a crossover or a non-crossover in one arm is equally 1/2 at 30°C with a respective diminution at 25°C when there is free crossing over along the entire chromosome. Under those circumstances we can observe crossing over in one arm while the same process goes on unhindered in the other one as well. This is shown in table 5 where values are also given for the expected and obtained proportions of simultaneous crossovers in both arms.

TABLE 5
Percentage crossovers in each arm and simultaneous crossovers.

NORMAL CHROMOSOME							
	LEFT ARM	PERCENT	RIGHT ARM	PERCENT	SIMULTANEOUS	PERCENT	EXPECTED
14°C	1713	48.7	1728	49.2	874	24.9	24.0
16.5°C	2846	49.6	2911	50.8	1455	25.3	25.2
25°C	2239	42.3	2424	45.8	1021	19.3	19.40
30°C	2103	46.6	2256	50.0	1060	23.4	23.3
INVERSION-BEARING CHROMOSOME							
	LEFT ARM	PERCENT	RIGHT ARM	PERCENT	SIMULTANEOUS	PERCENT	EXPECTED
14°C	1757	44.4	1518	42.8	642	18.1	19.0
16.5°C	2354	44.1	2442	45.7	1029	19.2	20.1
25°C	3527	37.5	4173	44.4	1563	16.7	16.7
30°C	2260	46.0	2433	49.5	1127	23.0	22.8

The fact that approximately 50 percent crossovers are obtained in each arm means that at 30°C the probability for getting a non-crossover in each arm is half, and the probability for a crossover also half. Actually this is another way of saying that the map distance of that arm is about 50 units. The probability of getting a non-crossover along the entire chromosome, that is, in both arms, will then be 1/4. The percentage values of tables 1 and 2 show that such is the case. It would also follow from the independence of crossing over in both arms that the probability of getting a simultaneous crossover in both arms should be approximately 1/4 at 30°C and the product of the respective probabilities for each arm at the other temperatures. The last two columns of table 5 give the obtained and expected percentages for simultaneous crossovers. The expected figures are calculated as the product of the actual crossover values obtained for each arm.

It is seen that for no temperatures is there any marked interference. Also at 30°C the obtained values approach 25 percent. The total diminution in crossing over for the inversion-bearing chromosome is again appar-

rent in the percentages of simultaneous crossovers which are lower than normal for all temperatures but 30°C.

A comparison of the total crossover values in the left arm which happens to be normal, given in table 4, with the normal left from table 5, shows that in the former case when there is no crossing over in the right arm the values are 45.8 percent at 16°C, 40.1 percent at 25°C, and 48. percent at 30°C and in the latter when there is free crossing over in the right arm we get 49.6 percent at 16.5°C, 42.3 percent at 25°C and 46.6 percent at 30°C. The same comparison can be made for the right inversion-bearing arm. Table 3 shows that when there is no crossing over in the left arm the values for the right are 44.4 percent at 14°C, 46.9 percent at 25°C and 51.3 percent at 30°C. Table 5 gives 42.8 percent at 14°C, 44.4 percent at 25°C and 49.5 percent at 30°C, when there is crossing over in the left. Taking the two sets together we are justified in saying that the values are not significantly different. Hence it may be concluded that whatever happens in one arm by way of crossing over has no influence on the other.

Cytological evidence (DARLINGTON 1932) indicates that the mechanism of crossing over occurs as shown in figure 1. It can thus be seen that chi-

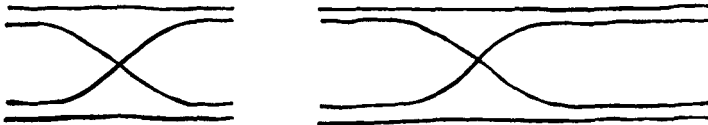


FIGURE 1.—Diagrammatic representation of crossing over. Only two strands cross at any given point.

asmatization, that is, in DARLINGTON'S sense, the coming together of two strands which have undergone interchange, must not necessarily be followed by genetic crossing over because only two strands chiasmata at one point. Genetic evidence (ANDERSON 1925, BRIDGES and ANDERSON 1925, REDFIELD 1930) had established that fact independently of cytological observation. Therefore when chiasmata takes place, the probability of a genetic crossover at that point is $1/2$ and that of a non-crossover also $1/2$. On this basis we can correlate the crossover ratios shown by the data presented in this paper with DARLINGTON'S mechanism of chiasma formation. It should then follow that at 30°C chiasmata must occur on the average at least once in each arm of the second chromosome, because the probability for a crossover in that region is $1/2$. At 25°C the number of chiasmata in each arm of the chromosome should be less than at 30°C. The number of chiasmata in chromosome II should therefore be between 2 and 2.5 because of the raising of the minimum value by the few triples and quadruples. That figure is in agreement with DARLINGTON'S computation of a mean of over 2 chiasmata in chromosome II. Should this frequency of chiasmata be verified cytologically it would prove

that there is no chiasmation or crossing over between sister strands. While not proving DARLINGTON'S assumption that chiasmation occurs only after crossing over has taken place, it would prove that each chiasma results in a genetic crossover whether the crossed strand goes into the egg or not. It is assumed of course, that strands are distributed in a random manner. If DARLINGTON'S hypothesis that chiasmation always results in a potential genetic crossover is wrong, then this simple correlation of two chiasmata to one genetic crossover should not hold. By the preponderance of chiasmata over genetic crossovers other theories concerning the relation of chiasmation to crossing over can be tested.

If we next arrange the data of tables 8 and 9 of the previous paper to show the proportions of double crossovers in each arm of the normal and inversion-bearing chromosomes, we observe the following:

TABLE 6
Proportion of double crossovers.

NORMAL CHROMOSOME							
	LEFT ARM	PERCENT	RIGHT ARM	PERCENT	SIMULTANEOUS	PERCENT	EXPECTED
14°C	141	4.0	190	5.4	8	.22	.21
16.5°C	196	3.4	248	4.3	10	.17	.15
25°C	75	1.4	152	2.8	4	.07	.04
30°C	183	4.0	290	6.4	13	.29	.26
INVERSION-BEARING CHROMOSOME							
	LEFT ARM	PERCENT	RIGHT ARM	PERCENT	SIMULTANEOUS	PERCENT	EXPECTED
14°C	118	3.3	106	3.0	4	.11	.10
16.5°C	123	2.2	118	2.2	3	.06	.05
25°C	138	1.4	247	2.6	5	.05	.04
30°C	261	5.3	312	6.0	14	.29	.32

If we assume that crossing over is as likely to occur in one part of a chromosome as in another, say in the first half of an arm as in the second, then the probability of a crossover in each half arm should be $1/4$ at 30°C and the probability of doubles in each arm $1/16$ or 6.3 percent, because strands that chiasmated in the first half would still have a $1/4$ probability of chiasmating again in the second, were there no interfering forces. The probability of simultaneous double crossovers in both arms would then be $1/256$ or 0.39 percent. The last two columns of table 6 give the values for the obtained and expected percentages of simultaneous double crossovers in each arm. The numbers of flies are admittedly very small but we see that so far as this method goes there is virtually no interference at any temperature. No deductions concerning loop length can be made from that because we have no convenient point which divides the arm in halves. It can be

seen again that the values for the inversion-bearing chromosome are lower than those for the normal one.

It follows therefore that both arms of chromosome II are independent in crossing over when the arms as a whole are considered. We can check this statement by considering the cross given in table 8 (GRAUBARD 1932) as if performed for the regions *al-pr* and *pr-sp*, adjacent distances which cover approximately each arm. The following computations are obtained by treating the chromosome as if it carried only three genes—*al*, *pr* and *sp*. Hence known doubles between *al* and *pr* count as non-crossovers, and singles and triples as singles. The crossover values at 25°C are 35.3 percent and 42.0 percent respectively. The expected value of doubles is 14.8 percent and the actual figure obtained is 15.0 percent. Similarly at 30°C for the same cross the crossover value for the left arm is 41.4 percent and for the right 43.6 percent. The expected figure for doubles is 18.0 percent and that obtained is 18.2 percent. The same agreement is obtained for the normal chromosome. The cross reported in table 9 shows that taking the equivalent distances, coincidence for the two arms is $\frac{17.3}{17.6}$ at 25°C and $\frac{18.8}{18.8}$ at 30°C. These data show that no interference exists between the two arms.

We can carry our computation further and see whether by taking sections smaller than the whole arm, interference steps in. We can consider in the same manner the two adjacent regions *tx-pr* and *pr-L* in the normal chromosome (table 9). We are now dealing with central sections of chromatin the sum of which is a little over half the total length. The crossover value for the section to the left of the spindle fiber is 32.1 percent at 25°C and 35.7 percent at 30°C. For the section to the right of purple the values are 17.6 percent and 20.5 percent respectively. Coincidence at 25°C is $\frac{5.6}{5.6}$ and at 30°C $\frac{7.9}{7.3}$. Hence again no interference.

One more calculation is of interest. If we consider the distances *al-cn* and *cn-sp* in the normal chromosome, we have one section containing a spindle fiber attachment and a second one without a fiber connection. At 25°C the crossover values for *al-cn* and *cn-sp* are 41.7 percent and 42 percent and at 30°C 43.7 percent and 42.3 percent respectively. Coincidence at 25°C is $\frac{17.2}{17.5}$ and at 30°C $\frac{18.2}{18.4}$. We can take similar regions in the inversion-bearing chromosome, for example, *al-L* and *L-sp*. At 25°C the crossover values are 39.2 percent and 35.7 percent and coincidence is $\frac{13.3}{14.0}$. At 30°C the crossover values are 45.9 percent and 36.0 percent and coincidence = $\frac{16.7}{16.5}$.

These data together with those given in table 6 seem to show that if we take sufficiently large, adjacent regions there is no interference. They also show that the presence of a spindle fiber introduces no modifications. Coincidence in a V-shaped chromosome such as the second with a spindle fiber attachment in the middle seems to be dependent upon the lengths of regions rather than the spindle fiber mechanism.

However, the presence of the spindle fiber does seem to exert some influence. Two small adjacent regions on either side of it, although never

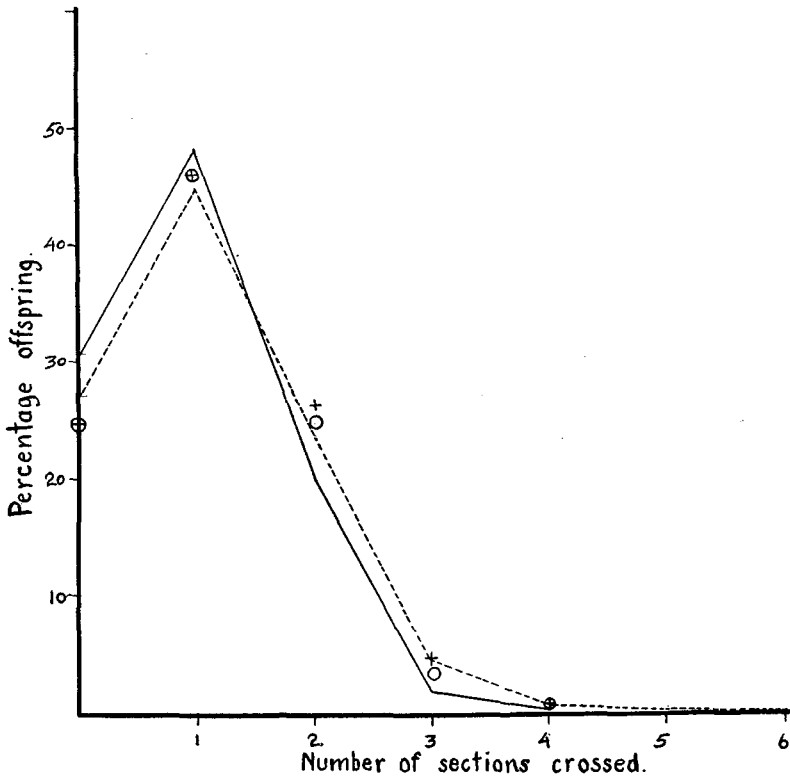


FIGURE 2.—Temperature effect on the frequency distribution of crossovers in the normal second chromosome of *Drosophila melanogaster*. Smooth line joins points obtained at 25° C, broken line at 30° C. Corresponding values at 14° C and 16° C are marked by 0 and + respectively.

showing a coincidence of one, show less interference than equivalent adjacent sections situated in one arm. Also regions lying very close to the point of spindle fiber attachment show an increase in their crossover values at the effective temperatures.

As shown in figures 2 and 3 the effective temperatures always diminish the non-crossover and as a rule the single crossover classes at the expense of an invariable increase in the number of multiple crossovers.

The effect of temperature seems to be limited to raising the probability

of crossing over in one section or another. In the case of chromosome II the effective temperatures operate so as to raise the basic crossover probability for small sections adjacent to the point of spindle fiber attachment. There is however no measurable influence on coincidence. What occurs is that due to the effective temperatures the probability of obtaining a genetic crossover in the regions adjacent to the spindle fiber is raised but

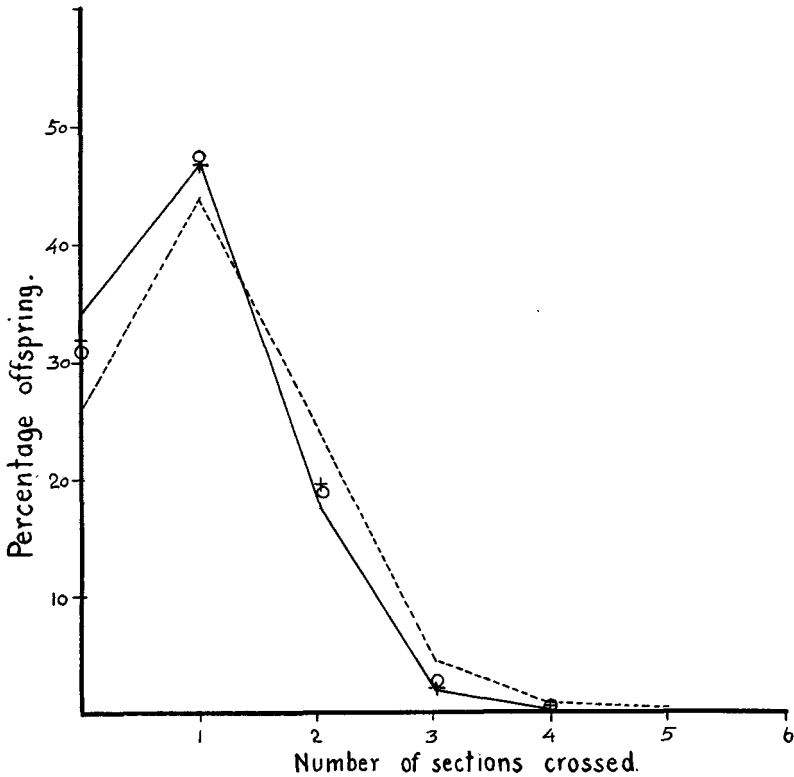


FIGURE 3.—Temperature effect on the frequency distribution of crossovers in the inversion-bearing second chromosome of *Drosophila melanogaster*. Smooth line joins points obtained at 25° C, broken line at 30° C. Corresponding values at 14° C and 16.5° C are marked by 0 and + respectively.

the probabilities for the further sections remain unaffected. The independence of the two sections still remains unaltered and the actual number of doubles is increased by exactly as much as should be expected on the basis of an increased frequency in one region only. This is demonstrated by the fact that the expected value, which is the product of the raised probability and the old probability for the unaffected section, is always in agreement with the figure actually obtained.

On the basis of DARLINGTON'S theory the number of chiasmata at 30°C should therefore be higher in the regions near the spindle fiber yet identical

for all temperatures in more distal sections, and the number of double chiasmata in each arm should be increased only in proportion to the rise in frequency of the central sections.

Whatever theory of crossing over is assumed the genetic evidence presented here seems to indicate that crossing over is so to speak, a property of a section of chromatin. For a given section there is a definite frequency of chiasmata with a determined frequency of genetic crossing over. According to DARLINGTON'S theory the ratio of chiasmata to genetic crossovers is 2:1. By observing the frequency of chiasmata that theory can be tested. If we take a section of the proper length, we get independence of crossing over between one section and another. It should therefore follow that crossing over is not a central mechanism for chromosome II but a local one, and that one half of it would be equally independent of the other half in crossing over even were there no spindle fiber dividing them.

Similar results showing the absence of interference in sufficiently large sections, such as the two arms of chromosome II, are obtained if the data are calculated on the basis of crossings over instead of crossover flies, that is, when a double crossover is counted as two crossings, a triple as three, et cetera. We then find that the proportions of crossings over in the left arm are 44 percent at 25°C and 50 percent at 30°C. The values for the right arm are 49 percent at 25°C and 56 percent at 30°C. The percentages of crossings involved in the simultaneous crosses as calculated from the data are 40 percent and 52 percent at the two respective temperatures. The values expected on an independent basis are 42 percent and 56 percent.

The average percentage of crossings over in each arm of chromosome II is 46 percent, or 0.46 crossing per fly at 25°C. For the total chromosome it is 93 percent or 0.93 crossing per fly. For the whole X chromosome (BRIDGES and OLBRYCHT 1926) the respective value is 0.62 crossing per fly. Although the correlation between percentage crossings per fly is in perfect agreement with the lengths of chromatin as measured for the X, one arm and the entire chromosome II, it is doubtful whether such a comparison is fruitful. There is no reason to believe that equivalent lengths of chromatin have identical crossing over possibilities in different chromosomes.

It was noticed before (GRAUBARD 1932) that when small non-adjacent sections situated far apart are considered, as for example *al-dp* and *vg-L* or *al-dp* and *L-sp*, interference is always operating. This is not quite correct if we consider that in computing coincidence between non-adjacent regions, we include cases which show intermediate crossing over. These latter are bound to introduce errors since when they are involved we no

longer study the interaction between the two regions selected for observation but introduce disturbances known to take place.

To obviate this complication we can use only those data in table 9 (GRAUBARD 1932) which are relevant and disregard the others. In computing interference between *al-dp* (section 1) and *L-sp* (section 7) we must consider only four classes of flies: Non-crossovers, crossovers in region 1, crossovers in 7, double (1-7) crossovers. The total of the flies consists of the sum of these classes. If this procedure is followed we get the following coincidence values.

TABLE 7
Corrected coincidence values for various regions.

DESCRIPTION OF SECTIONS	SECTIONS	COINCIDENCE VALUES	
		25°C	30°C
Far apart. In different arms	1-7	.9	1.1
	2-5	.9	1.0
Central in one arm, extreme in the other	3-7	1.0	.9
	1-4	1.0	1.0
Central in one arm, distal in the other	3-5	1.1	1.0
	4-2	.8	1.0
Both sections in the same arm	4-7	.7	.8
	1-3	.5	.9
Both adjacent to the spindle fiber	3-4	.7	.5

We see from these figures that there is no interference between regions sufficiently far apart. The suggestion may therefore be made that the terms loop and interference are not applicable to sections further apart than the maximum loop length which is capable of exerting interference. The picture of a loop was postulated to account for interference. The concept of interference presumes a tendency to form a loop after one crossing has occurred. Hence when a crossover occurs in region 1 and another one in region 7 there is no reason to consider that a loop in the above sense. They are two events occurring simultaneously and apparently independently. It stands to reason that when crossings take place in regions adjacent to either one of the two, they will exert an influence. For this reason they are neglected in the above computation because only the two sections must be isolated for study if we wish to find the influence of a cross in one upon that in the other.

The fact that the same values are obtained at 30°C means that temperature does not modify that relationship. It is also seen that sections in the same arm always show interference and that two small sections on either

side of the spindle fiber show higher coincidence than two larger, adjacent sections in one arm.

I am indebted to Professor J. B. S. HALDANE of the Department of Zoology, UNIVERSITY COLLEGE, London, and to Mr. M. D. SCHWEITZER of the Department of Zoology, COLUMBIA UNIVERSITY, New York, for useful criticism and helpful suggestions.

CONCLUSIONS

1. On the basis of DARLINGTON'S theory of crossing over there must be on the average at least one chiasmation in each arm of the V-shaped second chromosome at 30°C.

2. The effective high and low temperatures decrease the percentage of non-crossovers and increase the frequency of multiple crossing over.

3. The effective temperatures raise the probability for getting a crossover in the small sections adjacent to the spindle fiber. They exert no influence on coincidence. The rise in multiple crossovers is due to the increase in the frequency of crossovers in the sections adjacent to the spindle fiber. The resulting increase in multiples is in good agreement with the magnitude expected on the basis of the probability values.

4. If large enough sections are considered, no interference obtains in chromosome II. Crossing over in one arm is independent of that in the other arm. No interference exists for any adjacent regions of large enough lengths regardless of whether they contain the spindle fiber toward the center or at extreme ends. The same independence holds true for non-adjacent sections sufficiently wide apart, provided we consider only flies relevant to the relationship under examination. For two sections situated in the same arm interference obtains.

5. If a section of chromatin of a certain length has a given probability of producing a crossover, then the term interference cannot be applicable to two crossovers further apart than the maximum interference-exerting, loop size. They will be two independent events occurring simultaneously. Events in the intervening regions may influence one section or the other and they must be accounted for.

LITERATURE CITED

- ANDERSON, E. C., 1925 Crossing over in a case of attached X chromosomes in *Drosophila melanogaster*. *Genetics* 10: 403-417.
- BRIDGES, C. B. and ANDERSON, E. G., 1925 Crossing over of the X chromosomes of triploid females of *Drosophila melanogaster*. *Genetics* 10: 418-441.
- BRIDGES, C. B. and OLBRYCHT, T. M., 1926 The multiple stock "X-ple" and its uses. *Genetics* 11: 41-56.
- DARLINGTON, C. D., 1932 Recent advances in cytology. London: P. Blakiston's Son and Co., Inc. pp. 1-542.

- DOBZHANSKY, T., 1930 Translocation of the third and fourth chromosomes of *Drosophila melanogaster*. *Genetics* **15**: 347-399.
- 1932 Translocations involving the second and the fourth chromosomes of *Drosophila melanogaster*. *Genetics* **16**: 629-658.
- GRAUBARD, M. A., 1932 Inversion in *Drosophila melanogaster*. *Genetics* **17**: 81-105.
- MORGAN, T. H., BRIDGES, C. B. and STURTEVANT, A. H., 1925 The genetics of *Drosophila*. *Bibliogr. Genet.* II, 1925.
- REDFIELD, H., 1930 Crossing over in the third chromosome of triploids of *Drosophila melanogaster*. *Genetics* **15**: 205-251.