CHROMOSOME STUDIES IN THE LILIACEAE

I. Chromatid and Chiasma Interference in *Trillium erectum* L.

by

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I. INTRODUCTION

Chiasma studies in Trillium were made, (a) so that more might be learned of the nature and behavior of chiasmata, and possibly of their origin, and (b) because it seemed probable that at least most chiasmata represented genetic cross-overs, and it was hoped that such a study would yield evidence on crossing-over which either could not be obtained from genetic studies or which might be of interest when compared with the results of such studies.

It is possible to distinguish each of the five bivalent chromosomes in Trillium at first metaphase of meiosis. Further, in the preparations used, the four chromatids of each could in most cases be traced with respect to one another, something which has been done previously in only a very few cases. Using cells which could be completely analysed, the following data were obtained: (1) chiasma frequency per cell; (2) chiasma frequency per bivalent; (3) average chromosome lengths; (4) proportions and lengths of the different types of chiasma pairs; and (5) variations in chiasma coincidence resulting from differences in the distance between the two regions concerned.

Since two of the preparations used had a much higher chiasma frequency than two others, it was possible to determine the effect of the factors producing this difference upon (1) chiasma distribution,

(2) chiasma coincidence, and (3) the proportions of the different types of chiasma pairs.

The bearing which these data have upon genetical and cytological problems in other organisms is discussed. The main problems are: (1) the nature of chiasmata, (2) how crossing-over takes place, and (3) the nature of interference and coincidence, including interference between bivalents ("competition").

(a) The mechanism of crossing-over

Hearne and Huskins (1935), working on the first meiotic division in Melanoplus, were the first to trace the space relationships of the four strands in any considerable number of bivalents. Sax (1936) taking these data and considering two adjacent chiasmata at a time, showed that at least four cytologically distinguishable types of configurations occur. These are represented diagrammatically in Figure 1 together with four others which have been observed during the course of the present investigation. Each of the eight types is represented in the figure in two ways. From

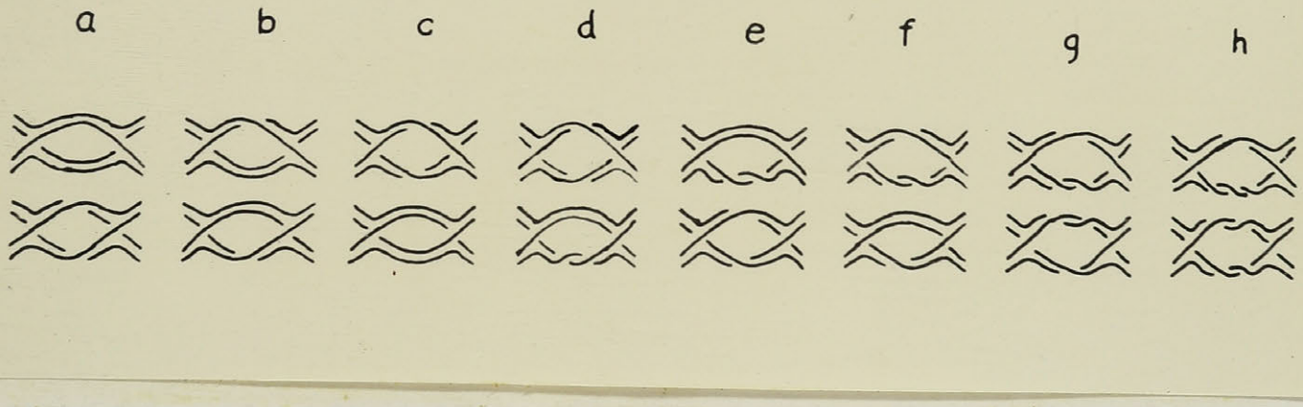

Figure 1. Diagrammatic representation of the eight cytologically distinguishable types of chiasma pairs observed in Trillium erectum. Each type is represented in two ways, with the two chiasmata in opposite directions (top row), and with the two chiasmata in the same direction (lower row). The two representations of each type cannot, of course, be distinguished cytologically.

a close examination of the drawings it will be seen that one representation of a given type could be converted into the other merely by moving two of the paired strands at one end relative to the other two. In a three dimensional model a given type might be represented as intermediate between the two. Therefore the two representations of a given type cannot be distinguished cytologically.

The difference between distinct types, however, can always be recognized cytologically, since no matter how much the configuration is twisted about, it cannot after it is once formed be changed from one type to another. This becomes evident on examining the figures.

On the assumptions of the current theories of crossing-over, one common feature of which is that chiasmata represent genetic cross-overs (Belling, 1933, Darlington, 1935, and Sax, 1936), certain definite proportions of these types of configurations would be expected. Sax found that the proportions observed in Melanoplus would not be expected on either of the other two theories, and suggested a modification of the Wilson-Morgan theory which would produce the different types in approximately the proportions observed. The data, however, were scanty and it seemed desirable that additional observations be made on some other suitable material, especially since terminalization occurs in Melanoplus. Our Trillium data suggest that the mechanism assumed in the Wilson-Morgan theory is also unlikely.

(b) Chiasma interference and coincidence

When a genetic cross-over takes place in a pair of chromosomes it renders less likely the occurrence of another cross-over close to itself. This phenomenon, known as interference, was first observed by Sturtevant in 1913. If one considers two adjacent regions on a chromosome, the simplest expectation would be that the frequency of simultaneous crossing-over would

equal the product of that in each of the regions separately. Usually simultaneous crossing-over in the two regions occurs less frequently than this; the phenomenon is known as interference. If the observed frequency of double crossing-over is divided by the expected, a value is obtained, usually less than unity, known as coincidence. Coincidence is the reciprocal of interference.

Without necessarily making the assumption that all chiasmata are genetic cross-overs, one can calculate the coincidence of chiasma formation in two regions of a chromosome. Haldane (1931) has shown that there is interference between chiasmata, similar, in terms of frequency distribution per bivalent, to that between cross-overs, but until now it has not been possible to show that chiasma interference and coincidence behave similarly in detail to the interference and coincidence of genetic cross-overs.

Variations in coincidence of chiasma formation along the length of the chromosome, variation due to differences in the distance between the two chiasmata, and variations due to factors causing an increase in chiasma frequency, have all been calculated. A review of the Drosophila data on coincidence of crossing-over will be reserved for the discussion.

(c) The nature of chiasmata

In a bivalent, stained to show internal structure, it may be seen that there are four strands or chromatids. At any level these four strands are associated in pairs. Frequently, in passing from one level to another, two of the strands exchange pairing partners. Such exchanges of partners are known as chiasmata, whether or not they appear as cross-formations, since this will depend upon the angle from which they are viewed. The interpretation of terminalized chiasmata (Darlington, 1932) is essentially similar, but this problem does not arise in Trillium.

There is definite cytological evidence that some chiasmata could only have arisen by breaking and rejoining of chromatids. Thus a number of chiasmata are definitely known to represent genetic cross-overs. There are many data suggesting that not only some but most chiasmata are of this nature (cf. Mather, 1938). There is, however, no evidence to show that all the chiasmata in any organism are genetic cross-overs, although this is commonly assumed. The present investigation has contributed additional evidence in favor of this conception in the striking similarity found between chiasma interference in Trillium and genetic interference in Drosophila.

(d) The nature of interference

The bulk of the available data on genetic interference has one limitation; it has been derived from the phenotypes of individuals into each of which only one chromatid from any bivalent has entered. Chromatid interference cannot be studied unless more than one of the strands of a bivalent are recovered. In females of most organisms only one of the products of a single meiosis functions as a gamete. In the males the four gametes of common origin usually become separated before maturity. This has limited the study of chromatid interference to organisms in which the products of meiosis remain together (e.g., the tetraspores of the mosses, and ascospores of certain fungi) and to the joined homologous chromosomes found in attached-X Drosophila females.

It is quite possible that the phenomenon of interference studied genetically is the result of two more or less independent forces, (1) chiasma interference, assuming that chiasmata are genetic cross-overs, and (2) strand or chromatid interference, that is a tendency for a strand involved in a cross-over at one level to take part in a cross-over at another level either more or less frequently than would be expected on a chance basis. It has been claimed by some (Beadle and Emerson, 1935, and Weinstein, 1936) that the second

of these two possible types of interference does not exist. Genetical evidence for the existence of chromatid interference has, however, been obtained by Lindegren and Lindegren (1937) in Neurospora and by Bonnier and Nordenskiold (1937) in Drosophila, coincidently with our report of its cytological counterpart in Trillium (Huskins, Newcombe, et al, 1937).

The present data and those of Hearne and Huskins (1935), assuming that chiasmata represent cross-overs, are the first in which it has been possible to test for chromatid interference by actual observation of the strands.

II. MATERIALS AND METHODS

Dr. S. G. Smith has very kindly loaned one of the best of his many preparations, and from it have been obtained the preliminary data for this paper. Two excellent preparations have also been loaned by Mr. G. B. Wilson (slides 1 and 2). These three slides were made in essentially the same way as my own preparations, the only difference being the temperature at which the material was kept previous to smearing.

Early metaphase smears of Trillium erectum were used in this study. The preparations were desiccated after smearing for from twenty to thirty seconds, and fixed overnight in La Cour's 2BD. Following this they were bleached with hydrogen peroxide in alcohol and stained in crystal violet according to a schedule essentially similar to that of La Cour (1931). In the later preparations, the washing before and after bleaching was reduced to a thorough rinse.

Spiralling of the chromatids in the few previously available slides of Trillium which showed really good structure, made it difficult to trace each of the strands through a bivalent without at some level confusing one with its pairing partner. However, by simply raising the temperature at which the Trilliums are grown it is possible to reduce the spiralling to a very considerable degree. By this method Trillium preparations may be obtained which show the individual chromatids and their positions relative to one another with a greater degree of clarity than have been obtained in any other organism of which we know.

It has not been possible in the past to obtain structure in material grown at temperatures higher than 18°C. Considering the success which we

have had this year with material grown in a large Drosophila incubator at temperatures as high as 25°, it would seem probable that previous failures were due to lack of ventilation in the closed temperature chambers used.

Wilson's preparations were made from material grown at 16°C. Some of the present preparations were made from material grown at approximately 20°C. (room temperature, 18°-22°) and some at 25°C.

No method was found which showed internal structure consistently in all preparations, or in all of the cells of the same preparation. Only a small proportion of the metaphase slides showed structure, and even in these the bulk of the metaphase cells had mass-stained chromosomes. The best slides each had about a dozen cells in which the space relations of all the strands could be determined with a high degree of accuracy.

Pre-treatment of smears with water, as recommended by Matsuura (1938), for periods of from one to five minutes, did not cause structure to become visible in either aceto-carmine preparations or in slides which were later fixed in 2BD. The application of water for one or two minutes between desiccation and fixation did not injure the structure already developed by desiccating. Desiccation in pure glycerine for one to two minutes produced ordinary mass-stained preparations showing only traces of the spiral structure.

Camera lucida drawings were made of twelve cells each from four slides. Slides 1 and 2 were those loaned by Wilson and were from material grown at 16°C. Slides 3 and 4 came from my own material grown at approximately 20°C. A magnification of 4100x was obtained using a 20x ocular and a Zeiss 120x objective. The total distance from the eye-piece of the camera lucida via the mirror to the paper was 15 inches.

III. OBSERVATIONS

Drawings were made showing the space relationships of the four chromatids at early metaphase. Eight cytologically distinguishable arrangements of chromatids were found in the chiasma pairs studied (see Figure 1). The proportions and lengths of these different types, and the positions of the chiasmata along the length of the chromosomes were determined. Complete cells were analyzed except in obtaining the preliminary data.

(a) Preliminary data

These were obtained during the year 1936-37 from a preparation kindly loaned by Dr. Smith. From this it was possible to determine the types of chiasma pairs and the distance between the two chiasmata in each pair. Due to the high degree of coiling, chromatids could be traced only in some of the bivalents of a cell, and frequently only through parts of a bivalent. In all, fifty pairs of chiasmata were analyzed. The proportions of the different types present among these are given in Table 2. Of the two main types, a and b, the average length of a was 2.3μ and of b was 3.3μ. That type a is significantly shorter than type b is shown by using a fourfold table for the two types and the numbers longer and shorter than the mean. The χ^2 obtained was 6.7, giving a value of $P \simeq .01$ (see Table 3). These were the data reported, together with those from Melanoplus, by Huskins, Newcombe, et al (1937).

(b) The main data

The main data were obtained this year from two slides loaned by Mr. Wilson (slides 1 and 2), and from two of those made by the author during the autumn of 1938 (slides 3 and 4). Slides 1 and 2 were from material grown at 16°C, 3 and 4 were from material grown at room temperature

(approximately 20°C). Slides 3 and 4 came from the same corm. From each of four slides twelve cells were drawn, and the space relationships of the chromatids in the five bivalents traced. In only a relatively few cases was there any confusion regarding the position of two chromatids relative to one another.

The data obtained are partly summarized in Table 1. In the 48 cells drawn thus there was a total of 1011 chiasmata. These formed 508 adjacent pairs, not including those straddling the spindle attachment. In 392 of these pairs it was possible to trace the positions of the chromatids. The eight different types actually observed are shown diagrammatically in Figure 1 and are arranged in order of their frequency of occurrence. It will be seen that the first four of these, a, b, c, and d, are those described by Sax (1936) as free, continuous, chromatid lock, and chromosome lock, respectively.

In Table 1 are given the chiasma frequencies, the number of pairs, the number of these in which the strands could be traced, and the numbers of each of the different types. Table 2 is taken partly from Table 1, and gives the average lengths of each of the observed types, in the preliminary work, the four preparations referred to above, and the work of Hearne and Huskins (1935) on Melanoplus.

(c) Pairs of chiasmata, types and lengths

It will be seen from Table 2 that the types of chiasma pairs which occur most frequently are, on the whole, the simplest, that is, those in which there is the least amount of twisting in the paired strands between chiasmata; and those which occur least frequently are the most complex. This general rule holds for all the data if it is assumed that the original forms of the chiasma

Table 1

Average lengths of the different types of chiasma pairs, and the data from which these were calculated. Twelve complete cells were analyzed from each slide

Slide	Chromosome	Number of Chiasmata	Number of Pairs	Pairs distinguishable	Type a			Type b			Type c			Type d			Type e			Type f			Type g			Type h		
					Number	Total Length	Average Length	Number	Total Length	Average Length	Number	Total Length	Average Length	Number	Total Length	Average Length	Number	Total Length	Average Length	Number	Total Length	Average Length	Number	Total Length	Average Length	Number	Total Length	Average Length
1	A	30	16	14	7	18.5	2.6	6	33.0	5.5	0	-	-	0	-	-	0	-	-	1	4.0	4.0	0	-	-	0	-	-
	B	52	30	25	15	37.0	2.5	6	20.0	3.3	1	4.0	4.0	2	5.0	2.5	1	2.0	2.0	0	-	-	0	-	-	0	-	-
	C	41	19	13	11	21.0	1.9	2	6.5	3.3	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
	D	65	40	30	20	40.5	2.0	7	17.5	2.5	0	-	-	3	12.0	4.0	0	-	-	0	-	-	0	-	-	0	-	-
	E	67	43	30	16	43.0	2.7	10	41.0	4.1	3	11.0	3.7	1	2.0	2.0	0	-	-	0	-	-	0	-	-	0	-	-
		255	148	112	69	160.0	2.3	31	118.0	3.8	4	15.0	3.8	6	19.0	3.2	1	2.0	2.0	1	4.0	4.0	0	-	-	0	-	-
2	A	36	24	11	4	9.0	2.3	7	23.0	3.3	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
	B	55	34	24	14	27.5	2.0	8	23.5	2.9	2	7.5	3.8	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
	C	45	18	11	6	10.5	1.8	5	16.0	3.2	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
	D	71	47	35	17	33.5	2.0	16	38.0	2.4	2	6.0	3.0	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
	E	69	45	25	12	26.5	2.2	10	36.0	3.6	2	7.0	3.5	0	-	-	0	-	-	0	-	-	1	10.0	10.0	0	-	-
		276	168	106	53	107.0	2.0	46	136.5	3.0	6	20.5	3.4	0	-	-	0	-	-	0	-	-	1	10.0	10.0	0	-	-
1 & 2		531	316	218	122	267.0	2.2	77	254.5	3.3	10	35.5	3.6	6	19.0	3.2	1	2.0	2.0	1	4.0	4.0	1	10.0	10.0	0	-	-
3	A	24	12	8	4	12.0	3.0	2	8.0	4.0	1	4.0	4.0	1	7.5	7.5	0	-	-	0	-	-	0	-	-	0	-	-
	B	23	7	7	5	23.5	4.7	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
	C	27	7	5	2	8.5	4.3	2	11.0	5.5	1	4.0	4.0	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
	D	39	16	12	6	20.5	3.8	5	21.5	4.3	1	2.0	2.0	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
	E	58	35	33	10	36.5	3.7	12	69.0	5.8	8	34.0	4.3	3	15.0	5.0	0	-	-	0	-	-	0	-	-	0	-	-
		171	77	65	27	101.0	4.1	21	109.5	5.2	13	55.0	4.2	4	22.5	5.6	0	-	-	0	-	-	0	-	-	0	-	-
4	A	24	12	12	2	11.5	5.7	6	28.0	4.7	1	2.0	2.0	2	8.0	4.0	1	2.0	2.0	0	-	-	0	-	-	0	-	-
	B	39	23	20	10	22.5	2.3	6	19.0	3.2	2	4.0	2.0	0	-	-	1	8.0	8.0	1	2.0	2.0	0	-	-	0	-	-
	C	30	10	9	3	9.0	3.0	6	17.5	2.9	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-	0	-	-
	D	45	23	22	8	22.5	2.8	9	29.0	3.2	4	12.5	3.1	1	1.0	1.0	0	-	-	0	-	-	0	-	-	0	-	-
	E	71	47	45	18	43.5	2.4	19	71.5	3.8	4	14.0	3.5	3	24.0	8.0	0	-	-	0	-	-	0	-	-	1	10.0	10.0
		209	115	108	41	109.0	2.7	46	165.0	3.6	11	32.5	3.7	6	33.0	5.5	2	10.0	5.0	1	2.0	2.0	0	-	-	1	10.0	10.0
3 & 4		480	192	173	68	210.0	3.1	67	274.5	4.1	24	87.5	4.0	10	55.5	5.6	2	10.0	5.0	1	2.0	2.0	0	-	-	1	10.0	10.0
1,2,3 & 4		1011	508	391	190	477.0	2.5	144	529.0	3.7	34	123.0	3.6	16	74.5	4.7	3	12.0	4.0	2	6.0	3.0	1	10.0	10.0	1	10.0	10.0

Table 2

Frequencies and lengths of the different types of chiasma pairs

	a	b	c	d	e	f	g	h	totals
				Frequencies					
Melanoplus (from Hearne and Huskins 1935)	27	26	9	3					65
Trillium (preliminary data)	33	15	1	1					50
Trillium (main data)	190	144	34	16	3	2	1	1	391
Total	250	185	44	20					
				Average lengths in microns					
Melanoplus	3.5	4.8	5.1	3.9					
Trillium (prelim.)	2.3	3.3	5.7	3.1					
Trillium (main)	2.5	3.7	3.6	4.7	4.0	3.0	10.0	10.0	
						5.4			

Table 3

Differences in length between types a and b, and significance as indicated by χ^2 and the nearest value of P. All measurements are in microns.

	type a		type b		Diff.in av.l.	χ^2	P
	number	av.l.	number	av.l.			
Melanoplus (H. and H.)	27	3.5	26	4.8	1.3	5.6	.02
Trillium (prelim.)	33	2.3	15	3.3	1.0	6.7	.01
Trillium (main data)							
Slide 1	69	2.3	32	3.8	1.5	14.1	.01
Slide 2	53	2.0	46	2.8	0.8	17.8	.01
(1 and 2)	122	2.2	78	3.3	1.1	20.3	.01
Slide 3	27	4.1	21	5.2	1.1	0.121	.70
Slide 4	41	2.7	46	3.6	0.9	3.9	.05
(3 and 4)	68	3.1	67	4.1	1.0	0.862	.80
(1,2,3 and 4)	190	2.5	144	3.7	1.2	6.6	.01

Note: χ^2 was calculated by means of a 2 x 2 table using the numbers of types a and b longer and shorter than the mean.

pairs were as drawn in the first row, that is, with the two chiasmata in opposite directions. If the configurations are drawn as in the second row of diagrams, with the chiasmata in the same direction, this relationship is only roughly true. The original direction of the chiasmata cannot be determined after opening out has occurred; the possible significance of this will be discussed later.

Further, the average lengths of the configurations (i.e., the distances between the two chiasmata) are least in the case of the configurations occurring most frequently, and greatest in the case of those occurring least frequently. (Types e, f, g, and h, are represented by very small numbers of observations and the lengths are not in accordance with this general rule. However, when the average for the combined four groups is calculated it is, as would be expected, somewhat higher than that of any of the less complex types.)

The significance of the difference in length between types a and b has been tested in the case of the preliminary data by means of a fourfold table, using the numbers of each type having lengths above and below the mean. This yielded a χ^2 of 6.7 showing that type b was significantly longer than type a (see Table 3).

Similar data from Melanoplus (Hearne and Huskins, 1935) have been included in Table 3. It was not expected that differences in lengths of different types would be as noticeable in Melanoplus chromosomes since it could be seen that the strands had in many cases become somewhat separated. Such a separation of the chromatids would tend to obscure the original positions of the chiasmata, and make determinations of the interstitial distances less accurate. There is no reason, however, to feel that any one type would be affected to a greater degree than any other. It was found that in chromosomes of Melanoplus type b configurations were longer than type a, just as in the preliminary Trillium data. The average length of type a was 3.5μ and of

type b was 4.8μ. A similar test of significance applied to these data gave a χ^2 of 5.6 showing that the greater length of type b was slightly significant statistically; P = .02.

In the main data the degree of significance of differences in length have been tested in a number of ways. Considering the first two slides alone, it will be seen from Table 1 that in each of the five bivalents from each slide, that is, in ten separate classes, type a is shorter than type b. The chance of this occurring by chance is one in $2^{10} = 1024$, that is, P = 0.00098. The same method when applied to the second two slides gave less consistent results. This was to be expected, however, since slides 3 and 4 had a much lower chiasma frequency than slides 1 and 2 and the numbers of observations in some of the chromosomes were necessarily very small.

The χ^2 test was also used as applied to the other data (see Table 3) to find the significance of the differences in length between types a and b. The data from slides 1 and 2 gave a χ^2 of 20.3, those of slides 3 and 4, a χ^2 of .862; P = < .01 and .80 respectively.

The low degree of significance in slides 3 and 4 needs a note of explanation. From Figures 3, 4, and 5 it may be seen that type a is only in excess in the lower size classes. In the higher size classes types a and b occur in more nearly equal proportions. As may be seen from Figure 2 the lower chiasma frequency in slides 3 and 4 has resulted in a sharp reduction of the number of chiasma pairs in the lower size classes, and a slight increase in the number in the higher size classes. Such a situation must necessarily have two effects: (a) to increase the average lengths of both types, and (b) to decrease the statistical significance of the difference in average length between the two types.

In order to determine the significance of the differences in length between all of the types as compared to one another, the standard error of the mean was calculated for each. The standard errors, and the significance of the difference, as indicated by P, are given in Table 4.

Because of the small number of observations in each of types e to h, these four have been grouped in making calculations.

It will be seen that type a is significantly shorter than any of the other types.

(d) Chiasma pairs across the attachment

Slides 3 and 4 were from material grown at a higher temperature than the other material. In them the region of the attachment was clearly split in some of the chromosomes. Ordinarily it is impossible to distinguish the type of a chiasma pair which includes the attachment in its interstitial region, owing to the impossibility of tracing the strands through the attachment. In sixteen of the chromosomes examined, however, both attachment regions were split so clearly that the strands could be traced through them. Of the chiasma pairs straddling the attachment, types a, b, c, and d occurred in the following numbers: 4, 10, 1, and 1, respectively (see Table 6). This is not a sufficiently large number from which to determine whether the attachment has affected the proportions of the types. In case there should be a noticeable difference between those in which one, both, or none, of the chiasmata are close to the spindle, the numbers are given separately in the table for each of these three classes.

Table 6 suggests that in chiasma pairs straddling the attachment the proportion of type a may be greatest where both chiasmata are adjacent to the attachment, and the interstitial distance short. This would be in agreement with the data obtained from the arms of the chromosomes. That is, the shorter

Table 4

Length differences between different types of chiasma pairs, standard errors of the differences, and significance of the differences in length as indicated by the nearest value of P. Types e, f, g, and h, have been grouped because of their small numbers.

types	difference of lengths in microns	σ_d	$\frac{\text{diff. of l.}}{\sigma_d}$	P
a - b	1.156	0.169	6.8	.000,000,001
a - c	1.107	0.265	4.2	.000,1
a - d	2.145	0.577	3.7	.000,1
a - (e,f,g,h)	2.918	1.224	2.4	.02
c - d	1.038	0.635	2.2	.02
b - d	0.989	0.601	1.6	.10
b - (e,f,g,h)	1.762	1.235	1.4	.16
c - (e,f,g,h)	1.811	1.251	1.4	.16
d - (e,f,g,h)	0.773	1.352	0.54	.59
b - c	0.049	0.314	0.016	.99

Table 5

Frequency distribution of different types of chiasma pairs in size classes

	Size classes in microns												
	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	Total
Slides 1 and 2														
Type a (free)	34	47	20	10	6	5								122
Type b (continuous)	4	22	19	12	13	3	2	2						77
Type c (chromatid lock)	1	1	1	4	3									10
Type d (chromosome lock)		2	2	1		1								6
Other types		1		1						1				3
Total	39	73	42	28	22	9	2	2		1				218
Slides 3 and 4														
Type a (free)	13	16	11	12	12	1	1	2						68
Type b (continuous)	3	8	20	17	6	4	3	2		2	1		1	67
Type c (chromatid lock)	1	7	5	3	4	3		1						24
Type d (chromosome lock)	1		2		2		2	2	1					10
Other types		2						1		1				4
Total	18	33	38	32	24	8	6	8	1	3	1		1	173

Table 6

Types of chiasma pairs straddling the attachment

	type *a*	type *b*	type *c*	type *d*
Both chiasmata adjacent to the attachment				
Slide 3	3	2	0	0
Slide 4	0	1	0	0
Combined	3	3	0	0
One chiasma adjacent to the attachment				
Slide 3	0	2	0	0
Slide 4	0	2	1	1
Combined	0	4	1	1
Neither chiasma adjacent to the attachment				
Slide 3	0	1	0	0
Slide 4	1	2	0	0
Combined	1	3	0	0
Total	4	10	1	1

the interstitial region, the greater the probability that the configuration is type a. However, it will also be seen that the proportion of type a is lower in each of these three classes than that found in the arms. Whether this is a real difference cannot be determined because of the small number of available data.

(e) Effect of crowding of chiasmata upon the types formed

It is of interest to note the effect which differences in chiasma frequency have upon the proportions of the different types of chiasma pairs. Fortunately, our slides showed considerable differences in chiasma frequency, 1 and 2 having an average of 22.2 chiasmata per cell, and 3 and 4 having an average of 15.8 per cell. It may be seen in Figure 2 that the greater chiasma frequency of the first two slides has resulted in a crowding of the chiasmata and an increased number of chiasma pairs with an interstitial length of between one and three microns. The data from which Figure 2 was derived are presented in Table 5. There is a slight but consistent decrease in the number of chiasma pairs with interstitial lengths greater than this.

It would be expected that since there is an increase in the number of chiasma pairs with short interstitial regions there would also be an increase in the proportion of type a, that is, the type which tends to have a short interstitial length. Such is the case, and in the cells from the two slides with the higher frequency the ratio of type a : type b is 122 : 77, whereas in those with the lower frequency it is 68 : 67 (see Table 1).

From figures 3 and 4 it appears that there is no consistent difference in the proportions of these two main types in similar length classes from slides having low and those having high chiasma frequencies. In the length class

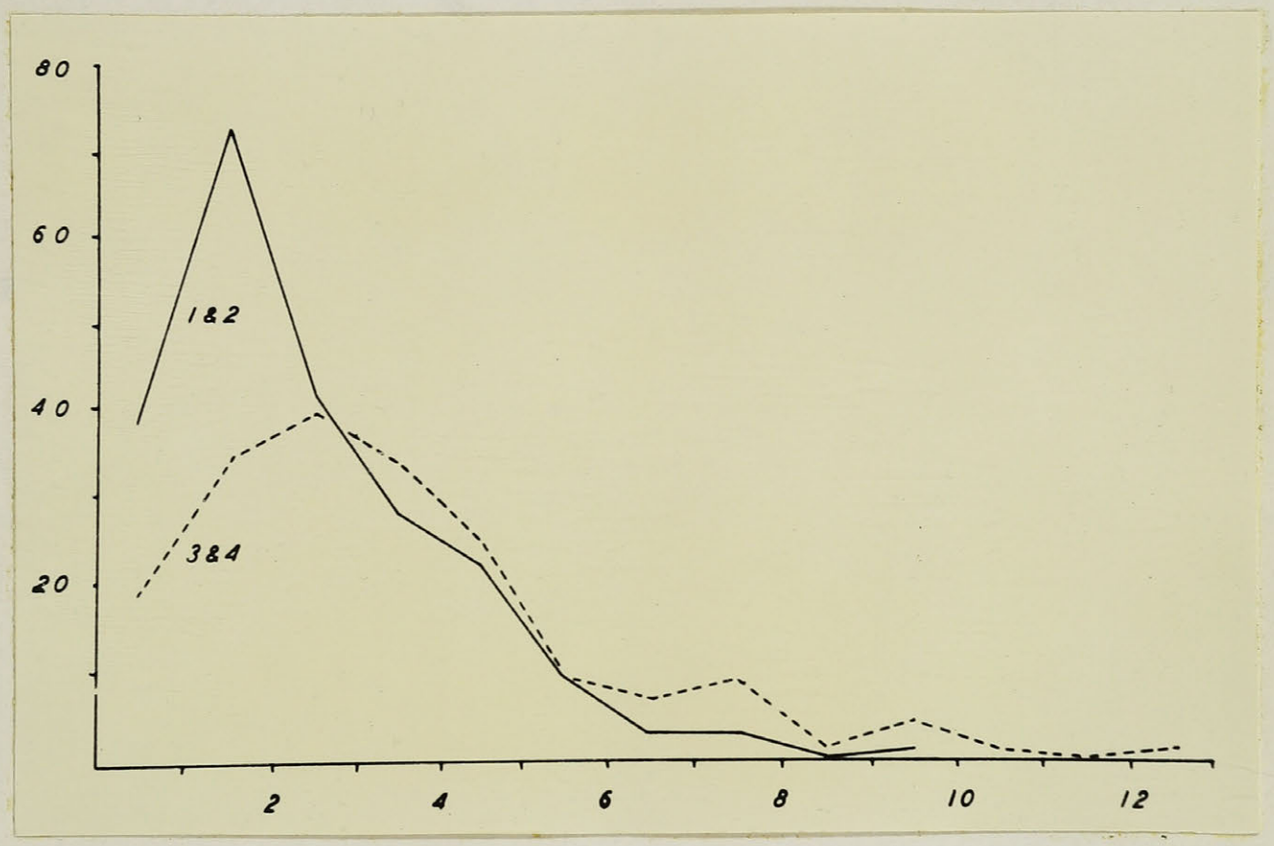

Figure 2. Frequency distribution of chiasma pairs of different lengths.
(From Table 5)

X axis: lengths of chiasma pairs in microns
Y axis: numbers of chiasma pairs in each size class

solid line: slides 1 and 2 (high chiasma frequency)
broken line: slides 3 and 4 (low chiasma frequency)

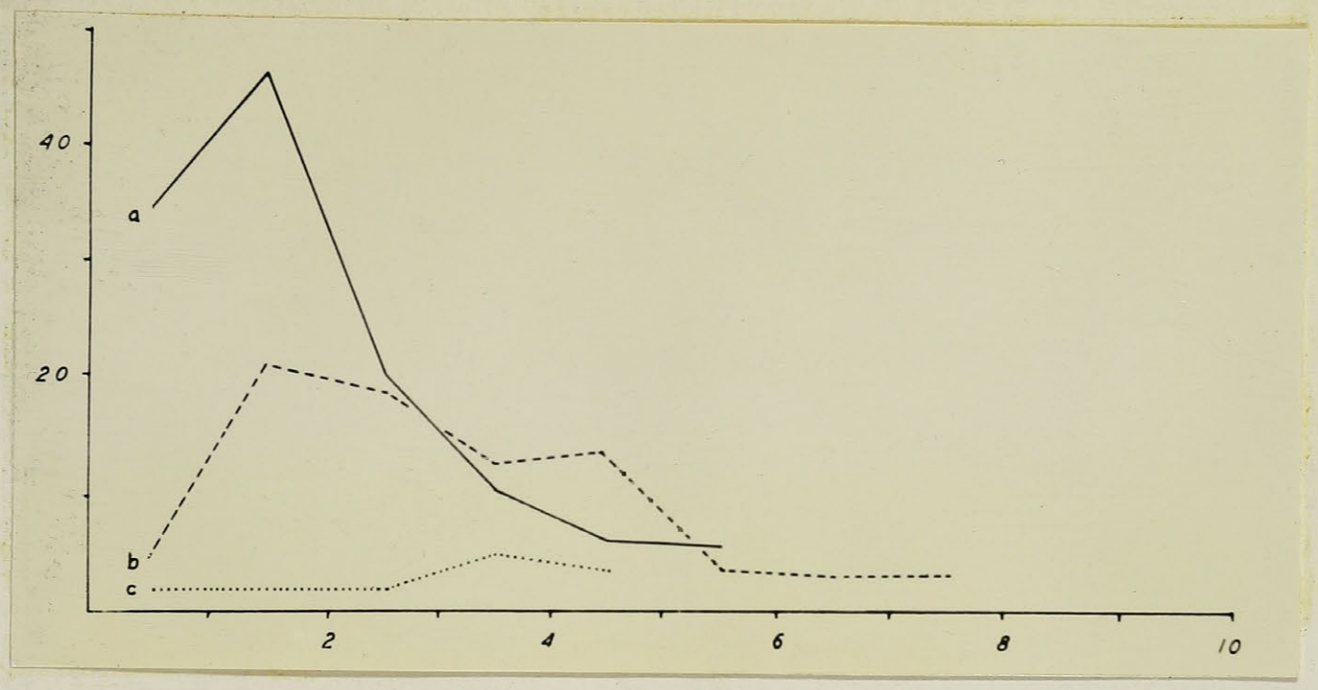

Figure 3. Proportions of types a, b, and c in the different size classes. (slides 1 and 2) (From Table 5)

X axis: lengths of chiasma pairs in microns
Y axis: numbers of chiasma pairs

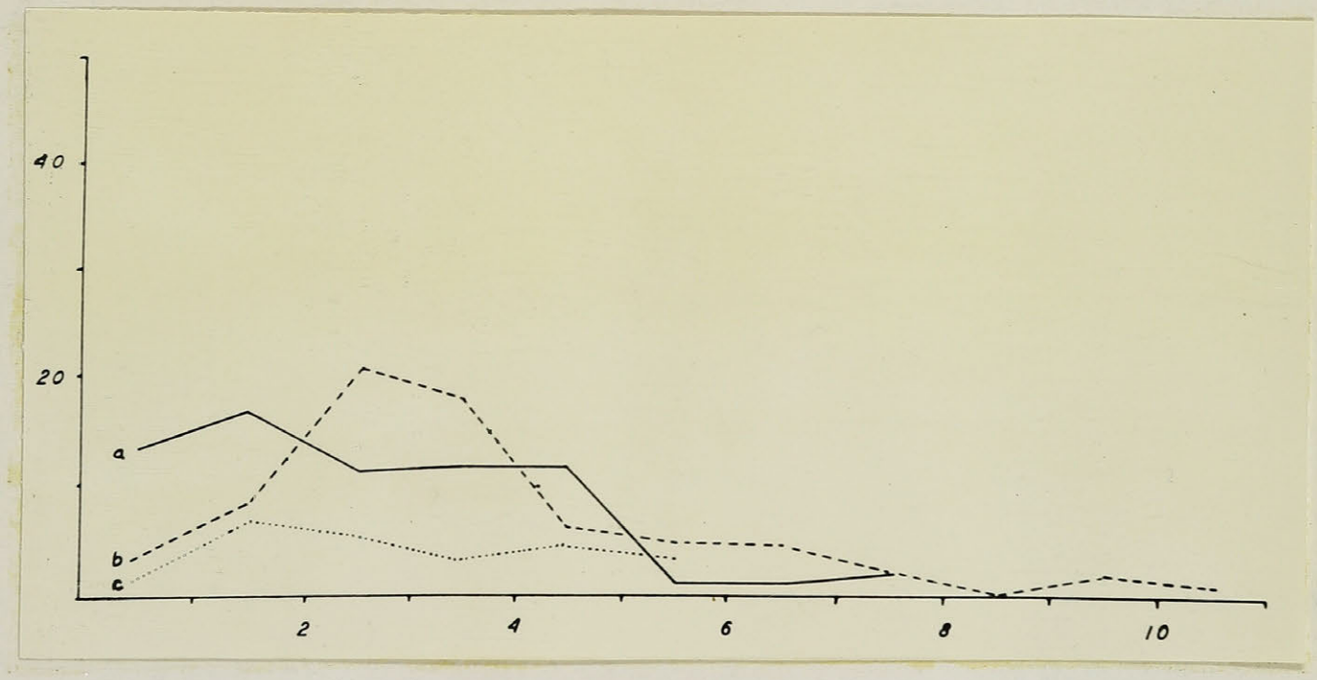

Figure 4. Proportions of types a, b, and c in the different size classes (slides 3 and 4) (From Table 5)

X axis: lengths of chiasma pairs in microns
Y axis: numbers of chiasma pairs

Note: One type b configuration in size class 12-13 was observed but has been omitted from the graph.

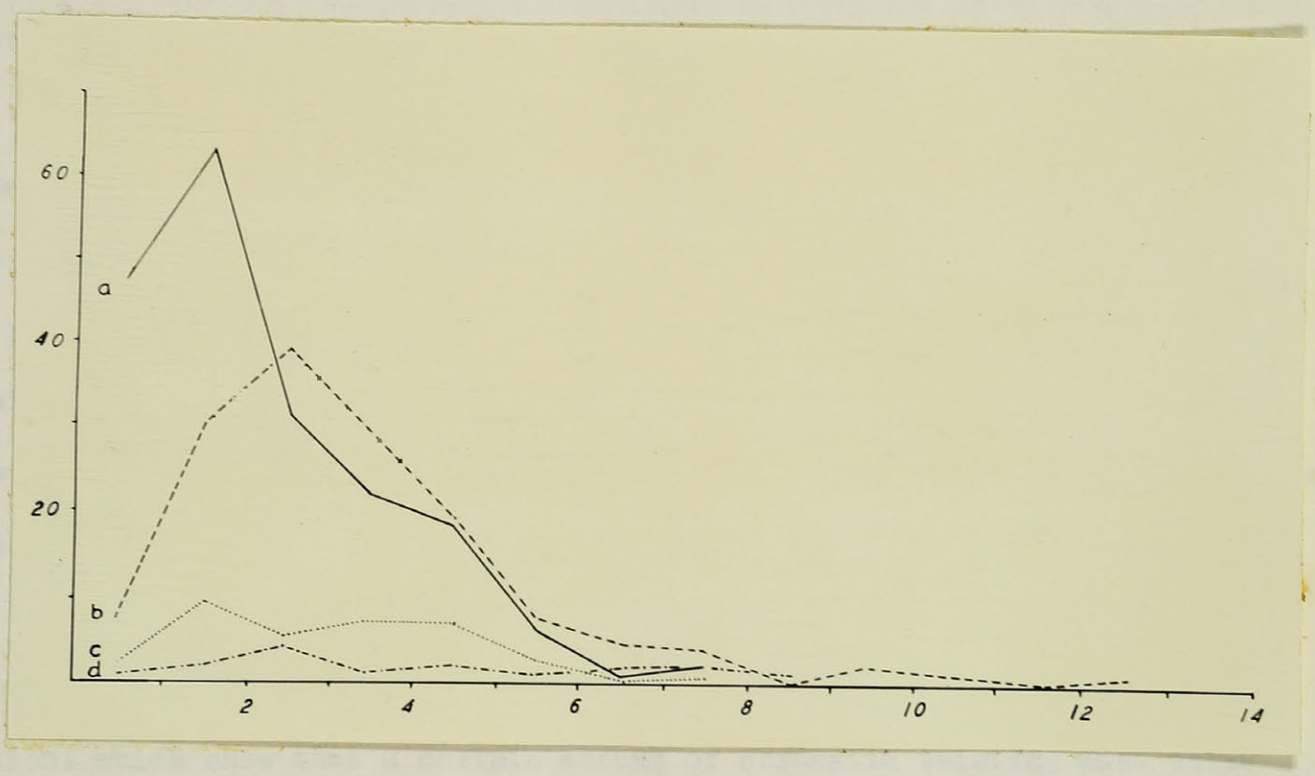

Figure 5. Proportions of types a, b, c and d in the different size classes (Slides 1, 2, 3 and 4 combined)

X axis: lengths of chiasma pairs in microns
Y axis: numbers of chiasma pairs

between one and two microns, type a is about twice as frequent as type b, both in the slides with a low and in those with a high chiasma frequency. Differences in proportions of types a and b do occur in the same size classes between the two groups of slides, but due to the small numbers representing these classes, these differences are not significant. For instance, the size class between one and two microns contains, in the case of both sets of slides, about twice as many type a as type b configurations. Other size classes, as for example the class from two to three microns, do not contain the same proportions in both sets of preparations, but there is no reason to believe that the difference is significant. Many more data would be necessary in order to demonstrate conclusively any difference in proportion between similar size classes in materials with high and low chiasma frequencies.

Without making any assumptions as to whether all chiasmata are genetic cross-overs or not, we may consider the possible significance of the observations which show that a certain amount of chromatid twisting must exist at the time of chiasma formation. The twisting could not have arisen later. It appears that if two chiasmata are formed a short distance apart there is less likelihood of chromatid twists occurring in the interstitial region than if the distance were longer. That is, when the chiasmata are a short distance apart a larger proportion of the simplest type, type a, would be expected than when they are a greater distance apart. This does not necessitate the assumption that the chromosomes are split and the halves twisted about one another prior to synapsis, since it is equally possible that they pair before splitting and that the plane of splitting rotates. Neither is it necessary to assume from these data that all chiasmata are genetic cross-overs, and that sister strands are paired at all levels after

crossing-over. However, this possibility will be discussed later.

(f) Chiasma distribution

The frequency of chiasma formation in different regions of the chromosomes of Trillium have been plotted separately for the slides having a high chiasma frequency (1 and 2), and those with a low frequency (3 and 4). The five chromosomes were divided into a number of regions each corresponding approximately to one micron.

The length of any particular chromosome varies considerably from cell to cell, and in order to get these data each chromosome had to be divided into a definite number of regions, regardless of its length in the particular cell being examined. The A chromosome, for instance, varied in length from 10 to 17μ. Since the average length was 12.2μ each individual A chromosome was divided into twelve equal regions regardless of its length. On a chromosome of average length each region would be 1.02μ long. Likewise in the other members of the complement, the regions were made to represent one micron, as nearly as possible, in an individual chromosome of average length. There was, however, one difference between the A chromosome and the others; the A chromosome has an almost terminal attachment region whereas the others have sub-median attachments. It was desired that the attachment separate two regions rather than occur within one. In order that this would be the case each arm was treated separately and divided into its particular number of regions. The points dividing the regions were numbered starting with the attachment, which was called zero. Thus the numbers indicate the distance from the attachment in microns, on a chromosome of average length. In Figure 6 are shown the number of chiasmata occurring in each of these regions. The data from which these were derived are given in Table 7. One striking feature of these graphs is the high frequency of chiasma formation in regions adjacent to the attachment.

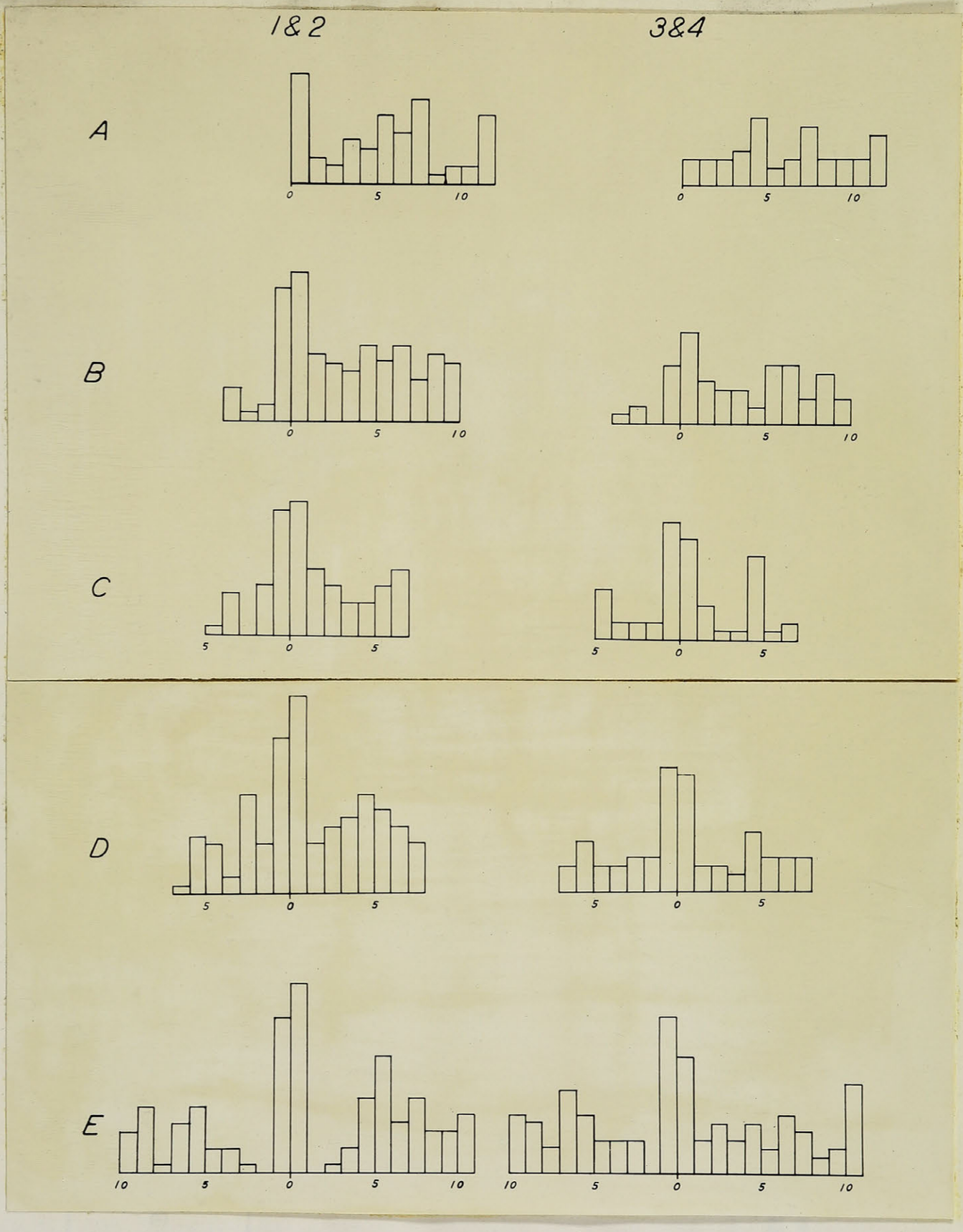

Figure 6. Frequency of chiasma formation in different one micron regions of the five bivalents of Trillium erectum. (Slides 1 and 2, and 3 and 4) (from Table 7).

X axes: one-micron regions in the chromosomes of Trillium numbered from the attachment which is called zero.

Y axes: relative frequencies of chiasmata (Actual numbers are given in Table 7).

Table 7

Distribution of chiasmata in the five bivalents of Trillium

Chromosome	Slides	Number of chromosomes examined	Regions of the left arm										Attachment	Regions of the right arm										
			10	9	8	7	6	5	4	3	2	1		1	2	3	4	5	6	7	8	9	10	11	12
A	(1+2)	24												13	3	2	5	4	8	6	10	1	2	2	8
A	(3+4)	24												3	3	3	4	8	2	3	7	3	3	3	6
B	(1+2)	24							4	1	2	16		18	8	7	6	9	7	9	5	8	7		
B	(3+4)	24							1	2	0	7		11	5	4	4	2	7	7	3	6	3		
C	(1+2)	22						1	5	0	6	15		16	8	6	4	4	6	8					
C	(3+4)	24						6	2	2	2	14		12	4	1	1	10	1	2					
D	(1+2)	24				1	7	6	2	12	6	19		24	6	8	9	12	10	8	6				
D	(3+4)	22				3	6	3	3	4	4	15		14	3	3	2	7	4	4	4				
E	(1+2)	24	5	8	1	6	8	3	3	1	0	19		23	0	1	3	9	14	6	9	5	5	7	
E	(3+4)	24	7	6	3	10	7	4	4	4	0	19		14	4	6	4	6	3	7	5	2	3	11	

Note: The divisions used were obtained by dividing the arms of each of the five bivalents into an even number of regions, in such a manner that in an arm of average length these would be as close as possible to one micron.

This is absent only in the A chromosome of slides 3 and 4. This exception may be due to an inversion found close to the attachment in the A chromosome of this plant.

It might have been expected in comparing the distribution of chiasmata in cells having a high frequency with those having a low frequency, that the reduction or increase would be greatest at the attachment, as is the case with crossing-over in Drosophila. With the exception of the A chromosome it would seem that the region near the attachment is less affected than the arm as a whole. As will be pointed out later this does not necessarily constitute a fundamental difference in behavior.

In the E chromosome, and to some extent in the D chromosome, it appears that on either side of the region of high chiasma frequency close to the attachment there are regions with exceptionally few chiasmata. These make it possible to classify the chiasmata, in the E chromosome particularly, as either adjacent to the attachment or distal to it, without any danger of confusing the two classes. It has been claimed by Matsuura (1938) that chiasmata adjacent to the attachment do not represent genetic crossing-over, but are rather the result of non-sister pairing between either the centromeres or the arms. It is not possible directly to disprove this, but it can be shown that his further assumption, randomness of pairing between sisters and non-sisters both at the attachment and in the arms, does not hold for our material. As Matsuura points out, randomness in this regard would result in a 2 : 1 ratio of types which he describes as "cross" and "parallel." The former type would be described according to our terminology as an arm having one chiasma adjacent to the attachment, and the latter as an arm having no chiasma adjacent to the attachment. The word chiasma is, of course, used here to denote a change in pairing partners, regardless of whether such a change results from breaking and rejoining of non-sister strands or from

alternate opening-out.

The E chromosomes from the two slides having a high chiasma frequency are particularly well suited for testing this, since, as mentioned above, chiasmata adjacent to the attachment are clearly distinguishable from others in the arm. Considering one arm at a time and using the data from these two slides (1 and 2), we have obtained a ratio of 42 cross : 6 parallel. Were this actually a 2 : 1 ratio, a chance deviation as large as that above would be expected only once in about 1000 cases. The data from the E chromosomes of the slides having a low chiasma frequency, also showed a deviation from a 2 : 1 ratio in the same direction, but the significance of this was somewhat less. These are summarised in Table 8. Chromosomes other than E were not used for this purpose since there was not the same clear distinction in them between chiasmata adjacent to the attachment and the other chiasmata in the arm.

(g) Chiasma frequency per unit length of chromosome

An attempt has been made to determine whether chiasma frequency is proportional to chromosome length when the length is sufficient for the formation of two or more chiasmata. A straight line relationship has been claimed by Mather (1934), Darlington (1937), Hearne and Huskins (1935) except in cases where localization was found or where the smaller chromosomes had to have more than a proportional number of chiasmata in order to insure maintenance of pairing. A straight line relationship when found in organisms in which individual chromosomes cannot be distinguished, is not proof that chiasma frequency is proportional to length when particular chromosomes are compared. In Trillium slides 1 and 2 did not show a straight line relationship (Figure 7). A more or less straight line relationship was obtained from

Table 8

Proportion of E chromosomes with chiasmata adjacent to the attachment

	two Xta. adjacent	one Xma. adjacent	no Xta. adjacent
Slide 1	9	2	1
Slide 2	10	2	0
Slide 3	6	4	2
Slide 4	8	3	1
Total	33	11	4

Ratios of arms with and without chiasmata adjacent to the attachment, deviation from a 2 : 1 ratio, and significance of the deviation as indicated by the nearest value of P.

	number of arms	adj. Xta.	no adj. Xta.	dev.	s.e.	dev./s.e.	P
Slide 1	24	20	4				
Slide 2	24	22	2				
Combined (1,2)	48	42	6	10	3.266	3.010	.001
Slide 3	24	16	8				
Slide 4	24	19	5				
Combined (3,4)	48	35	13	3	3.266	0.919	.36
Combined (1,2,3,4)	96	77	19	13	4.619	2.814	.01

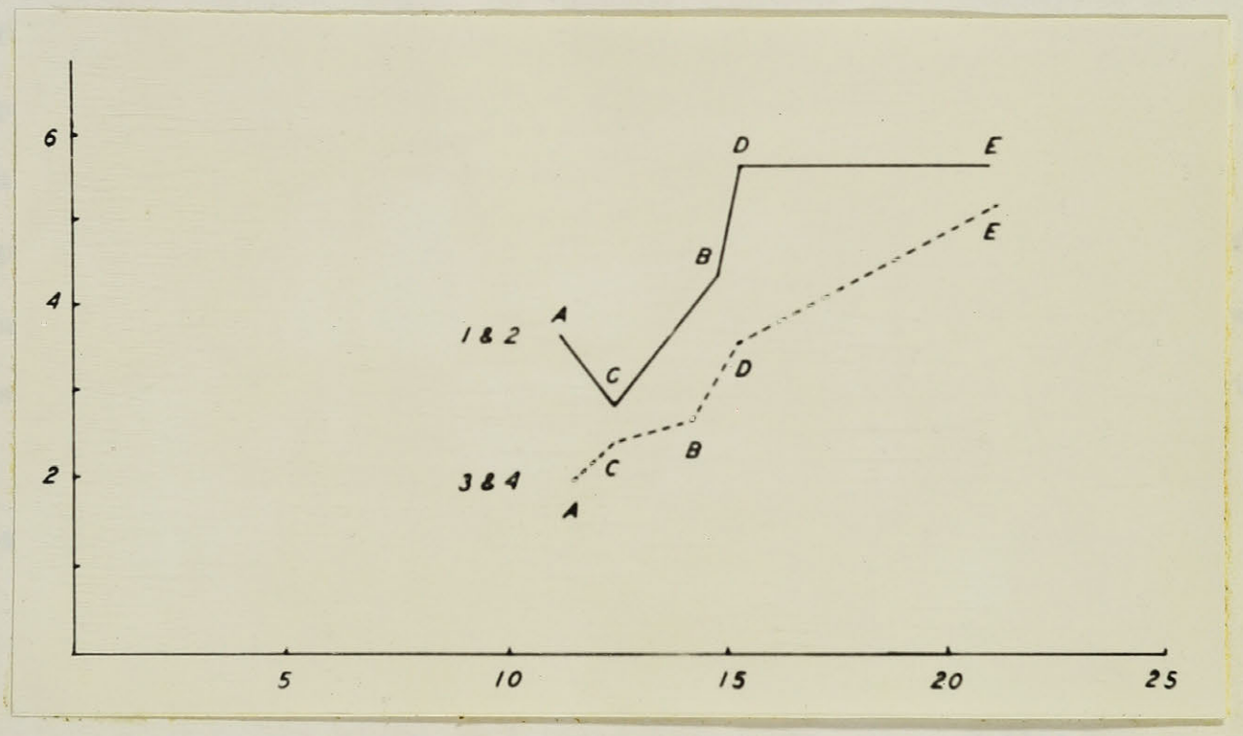

Figure 7. Relationship between chromosome length and chiasma frequency per bivalent. Slides 1 and 2 (high chiasma frequency) and slides 3 and 4.

X axis: average length of first metaphase chromosomes in microns
Y axis: average number of chiasmata per bivalent in twenty-four cells.

Note: Chromosomes A and D contain inversions in slides 3 and 4. Chromosome B has an inversion in slide 2.

slides 3 and 4 but it must be remembered that the two chromosomes, A and D, which show the reduction in frequency in these latter slides, making the graph nearly a straight line, both contain inversions. These inversions are in all probability responsible for the reduction. It would seem therefore that chiasma frequency in Trillium does not vary exactly as chromosome length. In fact the deviation from this appears to be considerable. The absence of proportionality cannot, as in other organisms, be attributed to localization resulting from failure of prophase pairing, nor can it be a genetical adaption to insure the pairing of short chromosomes, since were chiasma frequency proportional to length, the A chromosome, which is the shortest, would have fewer rather than more chiasmata. This is significant in relation to the cytological evidence on "competition" between bivalents, and will be discussed later.

(h) Coincidence of chiasma formation

In order to obtain values of chiasma coincidence in Trillium each chromosome of the complement was divided into a definite number of sections equal approximately to one micron in an individual chromosome of average length for its kind. The way in which this was done has been described previously.

If it is assumed that there is no terminalization in Trillium, it is possible to locate the position of all the chiasmata in a Trillium chromosome with a greater degree of accuracy than that with which genetic cross-overs can be located simultaneously in Drosophila. This is because it has been possible to divide the Trillium chromosomes into a large number of regions, from 12 for the A chromosome to 21 in the E chromosome. In studying genetic crossing-over the chromosomes cannot be divided into nearly as many regions

because of the inviability of individuals having too many mutant genes. Analysis has to be made of small regions at a time. Apart from possible movement of the chiasmata, which will be considered in the discussion, there is only one source of error in locating the position of a chiasma. That is that parts of a chromosome might be contracted to a greater or lesser extent than other parts in a particular cell. It is known, however, that chromonema length at a given stage tends to be very constant within a preparation (Wilson, unpublished). Most variation in general chromosome length at metaphase would therefore be due to differences in the amount of coiling. This being visible it is possible to see whether any particular part of a chromosome is more or less contracted than another part. It would seem that this is a negligible source of error when compared to the impossibility of determining the position of a cross-over within really narrow limits in Drosophila.

There is another advantage in direct observation of chiasmata. In Drosophila the marker genes cannot be spaced so as to provide regions of equal length either genetically or cytologically. Thus coincidence cannot be measured for two separate parts of a chromosome using regions the same distance from each other in both cases. It is highly desirable that the distance be the same in both cases since coincidence varies with the distance between the regions used.

This does not necessarily mean that we have measured coincidence of genetic crossing-over in Trillium by cytological methods, but it does mean that the measurement of chiasma coincidence and interference has been accompanied by fewer difficulties than exist in measuring genetic coincidence and interference in Drosophila.

Coincidence values were first calculated for adjacent regions, all the data from an arm being massed, and from it the average coincidence within that

arm calculated. Coincidence of chiasma formation in the two regions on either side of the attachment was calculated separately in each chromosome. The formula used was:

$$\text{coincidence} = \frac{x\ n}{a\ b}$$

in which x is the number of double chiasmata, n is the total number of observations, and a and b are the numbers of single chiasmata in the first and second region concerned.

Since there is a high frequency of chiasma formation close to the attachment sufficient data may be obtained from the two regions adjacent to it in any one of the five chromosomes to give a significant coincidence value. Within the arms, where the chiasma frequency is low, it is necessary to combine all the data from a comparison of each region with its neighbor, in order to get a significant coincidence value for that arm.

Coincidence values were calculated from slides 1 and 2, and from 3 and 4 separately. Values were obtained for each arm of a chromosome and across the attachment, using the one micron regions adjacent to one another. The data and coincidence values are given in Table 9. Table 10 summarizes these.

It is of interest to note that in all cases the coincidence values across the attachment are unity or greater, while those in the arms are less than unity. This is strikingly similar to the values obtained by genetic studies in Drosophila, and its significance will be considered later.

Another even more striking similarity is found when the data from slides with a high chiasma frequency (slides 1 and 2) are compared with those from slides with a low chiasma frequency (slides 3 and 4). As might be expected, coincidence is greater (that is, interference is less) in the arms of chromosomes from the high chiasma frequency slides. There is only one exception to this in seven cases and no reason to believe that the exception is any more

Table 9

Chiasma data and coincidence values for adjacent one micron regions

Chromosome	a	b	x	n	coinc.
High chiasma frequency (Slides 1 and 2)					
A	56	51	4	264	0.370
B (l)	7	19	0	72	0
B (a)	16	18	14	24	1.167
B (r)	77	66	17	216	0.723
C (l)	12	26	3	88	0.846
C (a)	15	16	12	22	1.100
C (r)	44	36	8	132	0.667
D (l)	34	52	8	144	0.652
D (a)	19	24	19	24	1.000
D (r)	77	59	17	168	0.628
E (l)	35	49	6	216	0.756
E (a)	19	23	19	24	1.043
E (r)	75	59	8	240	0.433
Low chiasma frequency (Slides 3 and 4)					
A	42	45	0	264	0
B (l)	3	9	0	72	0
B (a)	7	11	6	24	1.870
B (r)	49	41	4	216	0.430
C (l)	12	20	0	96	0
C (a)	14	12	9	24	1.286
C (r)	29	19	0	144	0
D (l)	23	35	3	132	0.419
D (a)	15	14	11	22	0.152
D (r)	37	27	2	154	0.308
E (l)	45	57	5	216	0.429
E (a)	19	14	13	24	1.173
E (r)	54	51	6	240	0.523

Table 9
(continued)

Chromosome	a	b	x	n	coinc.
Combined					
A	98	96	4	528	0.224
B (l)	10	28	0	144	0
B (a)	23	29	20	48	1.439
B (r)	126	107	21	452	0.704
C (l)	24	46	3	184	0.500
C (a)	29	28	21	46	1.190
C (r)	73	55	8	276	0.535
D (l)	57	87	11	276	0.612
D (a)	34	38	30	46	1.068
D (r)	114	86	19	322	0.624
E (l)	80	106	11	432	0.562
E (a)	38	37	32	48	1.092
E (r)	129	110	14	480	0.474

Note:- a and b are the numbers of single chiasmata in the regions compared; x is the number of double chiasmata in the two regions; n is the total number of observations. The capital letters denote the particular chromosomes, and (l), (a), and (r) signify that the regions compared were in the left arm, across the attachment, and in the right arm of the chromosomes respectively.

Table 10

Chiasma coincidence values between adjacent one micron regions in the left and right arms of the chromosomes and across the attachment. Taken from Table 9.

Chromosome	High chiasma frequency	Low chiasma frequency	Combined
A	0.370	0	0.224
B (l)	0	0	0
B (a)	1.167	1.870	1.439
B (r)	0.723	0.430	0.704
C (l)	0.846	0	0.500
C (a)	1.100	1.286	1.190
C (r)	0.667	0	0.535
D (l)	0.652	0.491	0.612
D (a)	1.000	1.152	1.068
D (r)	0.628	0.308	0.624
E (l)	0.756	0.429	0.562
E (a)	1.043	1.173	1.092
E (r)	0.433	0.523	0.405

than a chance variation. It is surprising, however, that exactly the opposite is true of the regions straddling the attachment and that in these the coincidence is less in the slides having the higher chiasma frequency. This behavior has been demonstrated by Graubard (1932) for coincidence of crossing-over in Drosophila and will be discussed later.

In the X chromosome of Drosophila it is found (Weinstein 1918) that coincidence increases, in general, as the distance between the two regions concerned. This relationship held up to the point at which coincidence reached unity or slightly higher, after which it was found to decrease slightly. Stephens (1936) claims that this subsequent decrease could have been the result of a faulty method used for calculating coincidence. The test of this length coincidence relationship in Trillium was made in the following way: Coincidence values were calculated for slides 1 and 2 and for slides 3 and 4, with the regions concerned separated by from zero to six other regions of approximately one micron each. The data from all the chromosome arms were grouped in order to obtain sufficiently large counts to give significant coincidence values at each of the different distances. Since it was desired to find the coincidence between two regions when there were no chiasmata in the intervening regions, all chromosomes having chiasmata in the intervening regions were disregarded in that particular comparison.

The data and the coincidence values obtained are given in Table 11. It may be seen that in general there is an increase in coincidence as the distance between the two regions increases. It seems quite certain that the increase continues until the coincidence exceeds unity. The two sets of slides disagree, however, as to whether or not there is a drop after this. Since the number of observations becomes smaller as the distance between the regions increases, less significance can be attached to the latter values.

Table 11

Coincidence values between chiasmata in regions separated by different distances. Units of measurement approximately one micron.

Slides	Distance between regions	a	b	x	n	coinc.
High chiasma frequency						
1 and 2	0	417	417	71	1540	0.629
	1	313	319	85	1016	0.865
	2	225	222	64	632	0.810
	3	130	127	38	383	0.822
	4	77	71	34	214	1.443
	5	35	32	14	94	1.175
	6	18	2	2	51	2.830
Low chiasma frequency						
3 and 4	0	294	304	20	1534	0.343
	1	253	254	41	1101	0.702
	2	185	200	39	735	0.775
	3	126	126	38	454	1.087
	4	73	71	20	270	1.042
	5	45	46	10	161	0.778
	6	28	26	7	83	0.798

IV. DISCUSSION

(a) Analysis of Existing Theories of Crossing-over and Chiasma Formation

During the past few years a number of theories have been put forward as to the mechanism by which chiasmata are formed. Since there is now a great deal of evidence that genetic crossing-over is accompanied by chiasma formation, these theories have been formulated to explain the two evidently related phenomena. They are further based on the appearance of the chromosomes at the stage during which chiasmata are believed to be formed. The first of those with which we are concerned was proposed by Belling in 1933. Belling, working on Lilium, observed that chiasmata could be seen after the splitting of the chromomeres into daughter chromioles, and postulated that the daughter chromioles might sometimes become joined to the neighboring chromiole on a homologous chromosome. The second theory, that of Darlington (1935), is based on the appearance of the chromosomes in the prophase nucleus of Fritillaria in which they seem to be coiled about one another. This theory postulates breaking and rejoining of homologous chromatids as the result of torsional strain. It is largely a deductive approach. Sax (1936) has recently brought forward and developed a torsion theory proposed originally by Wilson and Morgan in 1920. On each of these hypotheses certain cytologically distinguishable chromatid configurations would be expected in pairs of chiasmata. Sax has shown that the proportions of these different configurations expected on the Wilson-Morgan theory, though far from a good fit, are nearer the proportions which have been observed than those expected on either of the alternative theories.

In the present work more data have been obtained on the proportion of the cytologically distinguishable types of chiasma pairs. These data have been considered in relation to a further analysis of the above theories.

Since we are testing these theories on their own assumptions, one of which is that chiasmata represent genetic crossing-over, it is not necessary to know whether or not all chiasmata do represent genetic cross-overs in order to show that they are inconsistent with the observations on configurations of chiasma pairs.

(1) Belling's Theory

On Belling's theory chiasmata are conditioned by overlaps of homologous chromosomes. The chromomeres are supposed to split after the chromosomes are paired, and the original thread joining chromomeres of the same chromosome may go at random to either of the products of the division--the "chromioles." After the splitting a new thread will form between those daughter chromomeres, or chromioles, which are not already joined by the old thread. In cases where the original chromosomes overlapped, the new thread will join unattached chromioles by the shortest route. This will mean that chromioles from homologous chromatids rather than chromioles from sister chromatids will be joined in these cases. Belling states that there are two types of chiasmata possible on this theory, (a) those in which the strands which cross one another (these are the ones containing the original thread, and therefore they are genetically and structurally the non-cross-over strands) are on the same side of the group of four strands--the "direct" type of chiasma, and (b) those in which the strands which cross one another are diagonally opposite; this type is called "oblique." Examples of these types are

illustrated in Figure 8. This means that there are eight types of double chiasmata which may be formed and which will be expected in equal proportions. Four of these eight types are represented by types A, B, C, and D in the figure, and the other four are all similar to type E in that they have one direct and one oblique chiasma.

The proportions of the different types of configurations which are distinguishable at metaphase have been worked out in Figure 8. These are made on the assumption that types A, B, C, D, and E occur in the ratio of 1:1:1:1:4, and that in each of the chiasmata it is equally possible that strand a (or a') will pass over strand b (or b'), or that the opposite will be the case. This gives in all a total of 8 x 4 configurations. These are illustrated in the figure and the proportions of the seven distinct types possible are given. In Melanoplus it is not possible to distinguish between two of the three types of chromatid lock (types c and e) since there has been complete terminalization of one of the two chiasmata in most of the cases. The figures resulting from complete terminalization of one chiasma are identical in chromatid lock (a) (type c) and chromatid lock (b) (type e). Chromatid lock (c) has not been observed. Calling these by the letters used to designate the observed types in Trillium, types a, b, c, d, e, and f would be expected to occur in the following proportions: 5, 12, 6, 2, 2, 4 respectively. Another type, not observed, would be expected to occur once. The present data and those of Hearne and Huskins (1935) when compared with the expected percentages serve to test this theory.

On comparing these expected proportions with those actually observed in Trillium and Melanoplus it will be seen that this theory does not fit the data at all. Each of the types occurs either to a considerably greater or a considerably lesser extent than expected.

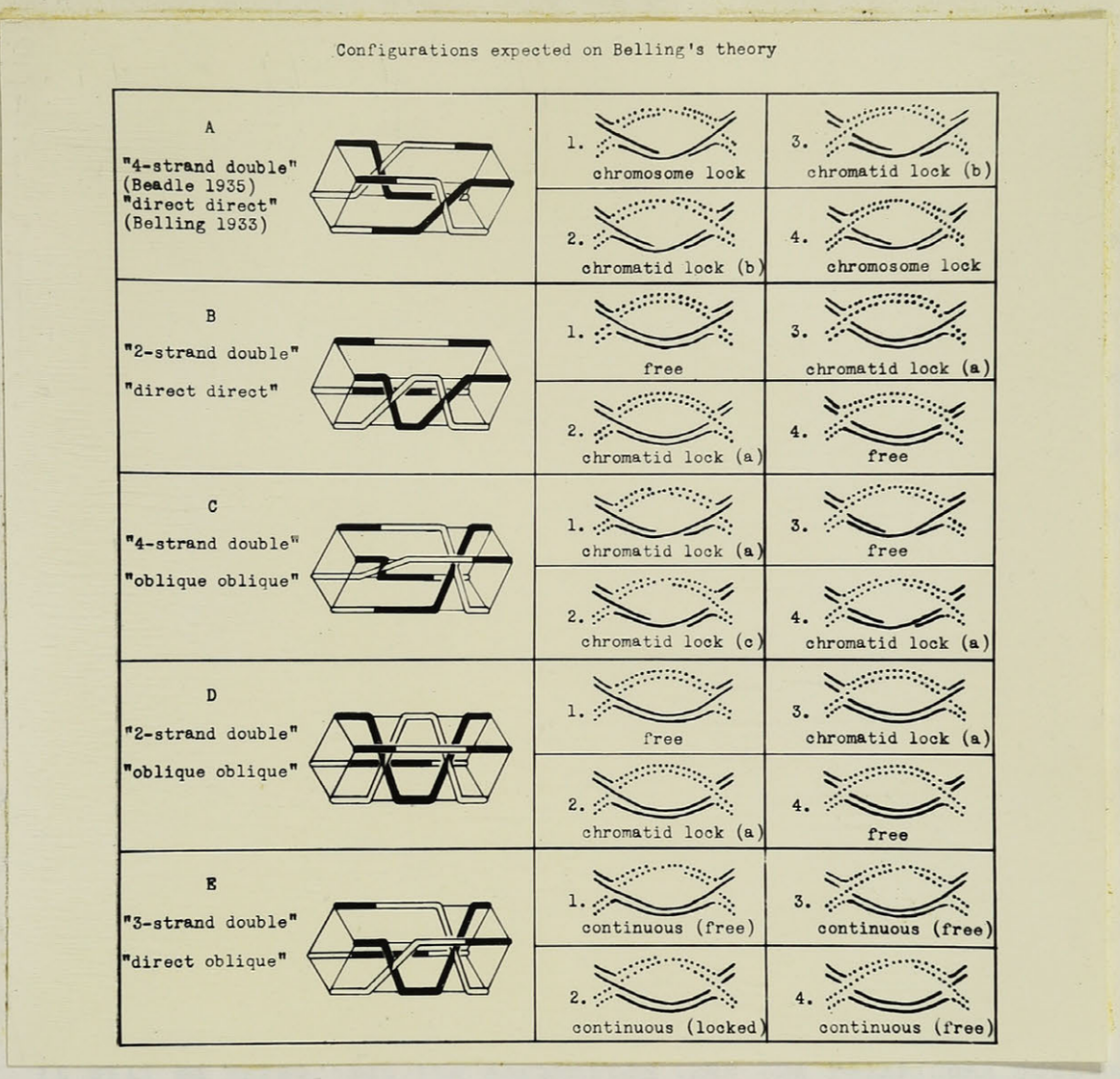

Figure 8. Proportions of types expected on Belling's theory. Types A, B, C, D, and E will be expected in the ratio of 1:1:1:1:4, making up the eight types described by Belling (1933). The four possible types containing one direct and one oblique chiasma produce identical configurations at diakinesis or metaphase and are therefore represented only once in the figure.

In those numbered 1, the black strand passes over the light strand in both chiasmata.

In those numbered 2, the black strand passes over the other in the first chiasma and under in the second chiasma.

In those numbered 3, the black strand passes under in the first chiasma and over in the second chiasma.

In those numbered 4, the black strand passes under in both chiasmata.

The following proportions of cytologically distinguishable types will be expected on Belling's theory:

free	(type a)	5	chromosome lock	(type d)	2
continuous	(type b)	12	chromatid lock	(type e)	2
chromatid lock (a)	(type c)	6	continuous (locked)	(type f)	4
			chromatid lock (c)	(not observed)	1

(2) Darlington's Theory

According to the theory proposed by Darlington, breaking and rejoining of the chromatids to form chiasmata is due to torsional strain. This is the result of the position assumed by the chromatids at late pachytene. It is supposed that the chromosomes coil around one another before each splits into two chromatids. It is known (Belling 1931) that the chromosomes elongate during the period when they are pairing, and it is assumed by Darlington (1935) that this is due to an uncoiling of an internal molecular spiral. "This uncoiling," he proposes further, "should lead to the coiling of the paired chromosomes around one another in the opposite direction to that of their internal spirals, unless their attractions allow them to slip." Each chromonema is assumed to be coiled in the same direction ("relational coiling").

It will be noted that the assumption that the relational coiling is in a direction opposite to that of the internal molecular spirals necessarily means that these internal spirals are in the same direction in homologous chromosomes. The only alternative to this is the difficult one that the molecular spirals are such that they may be uncoiled in either direction. Darlington has realized this difficulty but does not consider it insuperable. After the chromosomes split the sister chromatids become coiled around one another in a direction opposite to that of the chromosomes (Darlington 1937, page 549). This results from the partial uncoiling of the chromosomes which would naturally leave the chromatids twisted about each other in the opposite direction, providing the plane of splitting does not rotate. The simpler assumption is that it does not; the alternative will be considered later. Both the

twisting of the chromosomes in this manner, and the similar but opposite twisting of the chromatids, is termed relational coiling. The use of this term should be confined to the precise regular arrangement which he postulates. Such an arrangement causes a torsional strain to be set up which tends to break the chromatids. Once one of the chromatids breaks the strain is released in both that chromatid and its sister by partial uncoiling, but an additional strain is thrown on the two homologous strands, and one of them breaks at exactly the same place that its homologue broke, because the strain is greatest there. Since the strands which break uncoil slightly before they rejoin, the process of chiasma formation relaxes the strain in that region. In the resulting cross-over the direction of the crossing strands in the chiasma, that is, whether right- or left-handed, will be the same as the direction of the original chromosome coiling, it being a relic of the latter. The strands that cross each other in the observed chiasma (the "crossing strands") will genetically be the non-cross-over strands. It is further postulated that the direction of the relational coiling of the chromosomes is constant in any one arm; it follows that the direction of the chromatid coiling would also be constant.

Sax (1936) has worked out the proportions of the different configurations expected on Darlington's theory. These are as follows: free (type a), 0; continuous (type b), 50%; chromatid lock (type c), 24%; chromosome lock (type d), 0; and complex lock, 25%. In both Melanoplus and Trillium striking deviations from "expectation" in each of these classes has been found. Most striking is the fact that type a occurs with the greatest frequency when it is not expected at all on the theory.

In this feature alone it is difficult to harmonize the theory with observation. If we consider the case of type a configuration we see that

there is a fundamental reason why the theory provides no simple mechanism for the production of all types observed. It may be seen from Figure 1 that the free (type a) configuration may be represented in two ways. In the first, the chiasmata are in opposite directions. Since, however, the direction of a chiasma is a relic of the direction of the original chromosome coiling, and since such coiling is supposedly constant in any arm of a chromosome, all chiasmata in that arm would be expected to be in the same direction. Darlington has suggested, however, that in exceptional cases a redistribution of coiling from one arm to the other would cause a change of direction of chromosome coiling within that arm, and that when this occurred chiasmata in such an arm would sometimes be in opposite directions. However, as many as three type a configurations resulting from four chiasmata have been observed in one arm of a chromosome in Trillium. If the spacial relationships directly after chiasma formation are similar to those of the first diagram in Figure 1 in the case of all four chiasmata, this could not be explained by redistribution. Redistribution of chromosome coiling from one arm of a chromosome to the other could produce only one change; this arrangement would require three changes.

We have not eliminated the possibility, however, that free configurations may, directly after chiasma formation, have space relationships similar to those of the second diagram of type a. In this the chiasmata are of the same direction. It will be noticed, however, that between the chiasmata the chromatids are coiled for one half gyre about one another in the same direction as the chiasmata. Before the chiasmata are formed the chromatids are assumed to be coiled, however, in the opposite direction to that of the chromosome, that is, if the plane of splitting is determinate in the direction expected. Since, on this theory, it is the torsional strain resulting from their coiled state which causes them to break, partly uncoil, and rejoin, it is

scarcely probable that they would assume a direction opposite to that which they originally possessed, though they might easily become almost completely uncoiled between chiasmata.

If we assume that the chromatids may retain a tendency, not only to uncoil, but to coil in the opposite direction, such configurations may be obtained. But on Darlington's theory, as originally stated, coiling equilibrium would not be reached in this position.

In order to fit this theory to the observations mentioned it is necessary to assume that the plane of splitting rotates. This does not necessarily mean that the cleavage surface is indeterminate. Were the plane of splitting completely indeterminate the direction of chromatid twists between chiasmata would be at random. It will be seen that types a, b, and d have, on the assumptions of Darlington's theory, chromatid coiling in the same direction as the chiasmata. These types are represented by 455 observations. Type c (44 observations) has no chromatid coiling. Types e, g, and h (5 observations) have chromatid coiling in opposite directions in the two chromosomes. Only type f (2 observations) has chromatid coiling consistently as would be expected if the plane of splitting does not rotate, that is, the twists are in the direction opposite to that of the chiasmata. In order to accept Darlington's theory we must assume that the plane of splitting rotates in a direction such as to produce chromatid coiling almost consistently opposite to that which would be expected on the simplest expectation, namely, that the plane does not rotate. Thus, since the origin of this theory of crossing-over, additional data have increased rather than decreased the number of necessary assumptions for which there is no observational evidence.

(3) Wilson-Morgan Theory

On the Wilson-Morgan theory which has recently been developed and

discussed by Sax (1936) the four strands present at late pachytene are twisted together with the direction of twisting the same for all the four chromatids in any one region. (We could assume without departing materially from the basic features of this hypothesis that actual twisting may not always exist but that the torsional strain within the four chromatids is the same as it would be were they twisted in this manner.) It is supposed that the chromatids all remain parallel, i.e., that all four strands twist coincidently. On this theory homologous or non-sister strands are broken by torsional strain and reunite in such a way as to relieve the strain. The expected frequencies of the cytologically distinguishable types have been worked out in Figure 9. Those expected if the direction of the torsional strain is the same at the place of formation of the two chiasmata, and those expected if the direction of the torsion at the place of formation of one of the chiasmata is opposite to that at the place of formation of the other chiasmata, have been worked out separately. The proportions of the different types will be found in Table 12. It will be noted that if torsion is always the same the hypothesis is completely unsatisfactory since the chromosome lock (type d) may not be produced under such circumstances. If torsion is at random (either the same or opposite at the two places of breaking and rejoining) the percentages of the different types are remarkably similar to those expected on Belling's theory. They do differ, however, in that on this theory only one type of chromatid locked configuration is expected, and also in that the percentage of free is somewhat higher. The hypothesis remains somewhat unsatisfactory, however, for the same reason as Belling's. That is, with torsion at random, one would expect one quarter of the continuous configurations to be of the locked type (type f) and with torsion always in the same direction one half of the continuous would be of this type. Since type f has been observed only twice in 506 observations we must conclude that

Configurations expected on the Wilson-Morgan theory

1st breaks	2nd breaks	torsion same (1,2)	torsion opposite (1,3)
A B	A B	chromatid lock (a)	free
	A_1B_1	continuous (free)	continuous (free)
A_1B	A_1B	continuous (locked)	continuous (free)
	A_1B_1	chromatid lock (b)	chromosome lock
AB_1	A B	continuous (locked)	continuous (free)
	AB_1	chromatid lock (a)	free
A_1B_1	A_1B	free	chromatid lock (a)
	A_1B_1	continuous (locked)	continuous (free)

Figure 9. Proportions of types expected on the Wilson-Morgan theory. Sister strands are A and A_1, B and B_1. The strands which are broken at the first and second points of crossing-over are indicated in the first two columns respectively. When the torsion is the same at the two points of breaking the ends of the broken strands to the left of the breaks tend to move in a clockwise direction. When torsion is opposite the broken ends to the left of the first break and to the right of the second break tend to move in a clockwise direction. The numbers 1, 2 and 1, 3 indicate the direction of torsion in these two situations respectively and refer to Sax' (1936) Figure E.

Table 12

Proportions of types expected on the Wilson-Morgan theory (from Figure 9)

		Torsion same	Torsion opposite	Torsion random
free	type a	1	2	3
continuous (free)	type b	1	4	5
chromatid lock (a)	type c	2	1	3
chromosome lock	type d	0	1	1
chromatid lock (b)	type e	1	0	1
continuous (locked)	type f	3	0	3

in the two organisms studied crossing-over cannot be the result of a mechanism such as that suggested if we assume either of the above conditions with regard to the direction of the torsion. If, however, we assume that torsion is always opposite at the two places of breaking no continuous locked (type f) will be expected on the theory, and the expected proportions of the other configurations will be nearer to those observed. It will be noticed, however, that in both of the organisms studied a higher proportion of free (type a) configurations have been observed than would be expected. By making one further assumption it is possible to obtain a somewhat better fit between observed and expected. Let us assume that there is chromatid interference such that a strand which shows a tendency to cross-over at one point will also show a greater tendency than its sister to become involved in the adjacent cross-over. If these two assumptions are made it can be seen that the expected frequency of type a will be increased. The proportions will then be in somewhat closer agreement with those observed but the differences would still seem to be significant.

It is interesting to note that this provides a better formal fit than that of any of the other theories. It is not at all satisfactory, however, and there is as yet no observational support for the basic assumption.

It has been suggested that on any torsion theory it is difficult to conceive of a mechanism by which the two chromatids will always break in exactly the same place. Darlington believes that they do so because the strain is greatest at that one place. When we consider the contorted position of the chromosomes at the time of crossing-over it is difficult to imagine that there will not be many exceptions, and that unequal crossing-over will not take place at least frequently enough to be detected. This difficulty may possibly be overcome by supposing that at the time of crossing-over there

is a strong attraction not only between sister chromomeres or chromioles but also between homologous chromioles, and that the latter are paired tightly enough to influence very definitely the position of the break in the homologous chromatid. Such an assumption is incompatible with Darlington's but not with the Wilson-Morgan theory or this modification of it.

(b) The Nature of Interference

Interference has been studied chiefly in Drosophila and was first observed in 1913 by Sturtevant. Muller (1916) and Sturtevant (1915) found that interference tends to decrease as the distance between the two cross-overs increases. Weinstein (1918) studied interference between a cross-over occurring at one end of the X chromosome and those occurring at various distances from it. It was noted that interference is complete for a short distance (about 10 units). At 40 units coincidence, the reciprocal of interference, exceeded unity and at still greater distances dropped below unity again. The increase in coincidence with distance occurs in the other chromosomes (Bridges and Morgan 1923) but the succeeding decrease has been reported only in the X chromosome. Stephens (1936) claims that this could be due to the method by which the calculations were made.

The only detailed study from the cytological angle was that of Haldane (1931) who reported interference in chiasma formation. In the absence of interference it would be expected that the frequency distribution of bivalents with different numbers of chiasmata would be in the form of a Poisson series.

The variance of a Poisson series, obtained from the sum of the squared deviations of individual points, equals the mean. The variances of chiasma frequency distributions are lower than their means, usually between one and one quarter, due to the small number of bivalents with chiasma frequencies much greater than the mean.

Although it is thus demonstrated that there is interference between chiasmata it has remained to be shown that this interference varies along the length of the chromosome in a manner similar to genetic interference, that it varies with the closeness of the two chiasmata, and that it is affected by factors changing the chiasma frequency in the same way that genetic interference is affected by factors changing the frequency of crossing-over. The present observations have demonstrated such a similarity.

In order that comparisons between genetic and cytological interference of the nature suggested above be valid it is necessary to use an organism in which there is no terminalization. There is no proof that all movement of chiasmata is absent in Trillium, but it is certain that terminalization, as such, has not gone on in our material. By terminalization is meant movement of the chiasmata away from the attachment regions, probably due to a repulsion between the two attachments causing them to separate. Terminalization cannot have taken place in our material since a very high chiasma frequency exists close to the attachment region. There is an alternate possibility, however, which is that some chiasmata have moved toward the attachment, as found by Hearne and Huskins (1935) in Melanoplus. Although this cannot be disproved it would seem improbable in Trillium. It has been shown that the chiasma coincidence across the attachment is considerably in excess of unity. That is, if one chiasma is present close to the attachment on one side there is a

greater possibility of another occurring close to the attachment on the other side. If these adjacent chiasmata were not formed in this position but were moved there by the attraction of the attachment region, it would be necessary to assume that some of the attachments exerted a greater attraction for chiasmata than others. Further assumptions would be necessary in order to explain the behavior of coincidence in preparations having different chiasma frequencies. The possibility that the chiasmata observed adjacent to the attachment were not formed in that position but moved there cannot be disregarded, but it is at least much simpler to assume that chiasma movement is negligible and that the chiasma coincidence values obtained are similar to those which would be obtained were it possible to test for them genetically in this organism. The similarity of behavior of chiasma coincidence and genetic coincidence would lend support to this view.

The behavior of cytological interference and coincidence in Trillium is, as has been shown, strikingly similar to genetical interference and coincidence. The simple relationship between interference and length of chromosome separating the cross-overs found by Sturtevant (1915), Muller (1916), and Weinstein (1918), apparently holds for chiasmata. Chiasma coincidence increases as the distance between the chiasmata up to a certain point at which it is somewhat in excess of unity. Beyond that point the data from the two separate sets of slides are too few to show any trend with certainty. As in the case of Weinstein's data there is no absolute certainty as to whether or not coincidence again drops below unity. The agreement therefore between these two phenomena is as complete as could be expected.

Distribution of chiasma coincidence along the length of the chromosome is also similar to that found in the chromosomes of Drosophila having two arms. Bridges and Morgan found that the region of highest coincidence for the second

chromosome was purple (54.5) and for the third chromosome was pink (48.0). These genes are located very close to the attachment region. It may be said in general that in Drosophila coincidence is greatest across the attachment. In chromosome III coincidence reached a value as high as 1.3 in this region. It has been noted that this is also the case in the chromosomes of Trillium. Coincidence within the arms of the chromosomes was consistently lower than unity in each of the five bivalents and in both sets of preparations. Coincidence across the attachment was consistently higher than any values obtained within the arms, using regions of similar lengths.

The most striking feature of the behavior of coincidence in Drosophila is shown by Graubard (1932). It was found that when chiasma frequency was increased by rearing the flies at a high temperature coincidence was also affected. Comparing the 25° and 30° lots it may be seen that, in sections situated within the same arm, an increase in crossing-over was accompanied by an increase in coincidence. This was consistent in all of the twelve observations. In regions not of the same arm an increase in chiasma frequency was accompanied by a decrease in coincidence. This was consistent, with one exception, in each of the eighteen observations (see Graubard's Table 11). That a similar situation should exist in Trillium was most unexpected, but it seems unquestionable that differences in chiasma frequency similar to these have been accompanied by similar variations in chiasma coincidence. This difference between coincidence and its behavior in the arm of a chromosome and across the attachment, indicate certain peculiarities of the attachment region. The possible nature of these will be considered later in this paper.

So far we have dealt only with chiasma coincidence and have shown that it is extremely similar to coincidence of genetic crossing-over in Drosophila.

This similarity is so striking that it may be considered very strong evidence supporting the concept that all, or at least practically all, chiasmata represent genetic cross-overs. As yet there is no conclusive evidence on this point.

It has been shown in genetically marked chromosomes rendered cytologically distinguishable by means of translocated pieces of other chromosomes, that genetic recombination is accompanied by recombination of the cytologically marked ends. (Creighton and McClintock (1931) in corn, and Stern (1931) in Drosophila.) In these studies no observations could be made on chiasmata.

The evidence that chiasmata are accompanied by genetic crossing-over has come from the observation of interlocking bivalents and of configurations observed in polysomics, some of which could have arisen only by breaking and rejoining of chromatids. The list of authors who have observed interlocking bivalents is too long to be included in this paper. Of the second type of evidence (that is, configurations found in trisomics and tetrasomics) trivalent configurations were observed in Hyacinthus by Belling (1929) which could not be explained without assuming that the strands had broken and rejoined where the chiasmata occurred. A quadrivalent was also found by Darlington (1930) which was similar in that it too necessitated the assumption that chiasmata were the result of breaking and rejoining of the chromatids. The proof hinges upon the assumption that prophase association takes place only in two's. Recent investigations in corn would seem to supply the necessary evidence on this point.

There is still no proof that all chiasmata are genetic cross-overs, but evidence showing that chiasmata behave in a manner similar to genetic cross-overs can be interpreted as indicating that most, if not all, chiasmata are of this nature.

The evidence of this type has been reviewed by Mather (1936) and Darlington (1937) and need not be dealt with in detail here. To the evidence for the chiasmatype hypothesis may be added that presented earlier, that interference and coincidence between chiasmata in Trillium behave in a manner essentially similar to genetic interference and coincidence in Drosophila. The total evidence, therefore, although not conclusive is very strong.

If we assume that all chiasmata represent genetic cross-overs it is possible from our data to investigate another phase of the interference problem, namely chromatid interference.

It might be well to mention that caution should be exercised in interpreting the cytological evidence on competition between bivalents, and that since the data obtained on this point could have an alternative explanation it should not be used as evidence for the chiasmatype theory.

Competition or interference between bivalents has been investigated in two ways, (a) by testing for a negative correlation between the chiasma frequency of a particular cytologically distinguishable bivalent and the rest of the complement, and (b) in organisms with chromosomes of equal length by testing for a greater variability in chiasma frequency of bivalents of the same cell than would be expected on the basis of the observed intercellular variability.

The former method has been used by Sax (1935) on the M chromosome of Vicia faba, by Mather and Lamm (1935) on the same material, and by Mather (1936) on Oenothera. In none of these cases was there any significant correlation. The second method which is the only one giving significant results has one limitation. If the results are to be interpreted as indicating the presence of competition it is necessary to assume that the chiasma frequency is equal in chromosomes of equal length. Mather states

that "……it is known that, apart from very short bivalents, the mean chiasma frequencies of bivalents in any organism are approximately proportional to their lengths."

Obviously it is not possible to test this in organisms having chromosomes which are indistinguishable. In Trillium there is a distinct deviation from proportionality. Also, structural hybridity (inversions), such as has been observed in three chromosomes while studying preparations from only three Trillium corms, could result in a decrease in chiasma frequency in a particular chromosome. The distribution of chiasmata among the chromosomes resulting from lack of proportionality would on Mather's method be interpreted as competition. For this reason it is felt that the phenomenon observed cytologically is not necessarily parallel to the genetic phenomenon observed in Drosophila, and that the apparent similarity should not be used as evidence for the chiasma-type theory until the above alternative is eliminated.

1) Chromatid interference: random occurrence and recurrence of crossing-over

Weinstein (1936) in defining the problem states that:

"complete random association of chromatids in crossing-over implies (1) that at any given level any two chromatids of a tetrad are equally likely to cross-over (this may be termed random local association, or random occurrence of crossing-over); (2) that the two chromatids which cross-over at one level do not determine which shall cross-over at other levels (random recurrence of crossing-over).

He has concluded from analysis of Drosophila data that:

"(1) There is no crossing over between sister chromatids.
(2) At any level only two of the four chromatids may cross-over.
(3) Otherwise it is a matter of chance which chromatids cross-over at any level.
(4) The chromatids that cross over at one level do not determine which ones cross-over at other levels."

Complete random association of homologous chromatids also implies that the types of couble cross-overs known genetically as two-, three-, and four-strand doubles, will occur in the ratio of 1 : 2 : 1. These types are so named because two, three, or all of the four strands have become involved in at least one of the two cross-overs.

It will be observed that the three-strand doubles correspond to the continuous or non-compensating type observed cytologically (type b configuration also types f and g). The two and four-strand doubles cannot be distinguished cytologically and have collectively been termed compensating chiasma pairs. Were crossing-over occurrence and recurrence both at random, compensating and non-compensating pairs would be expected in equal numbers, assuming of course that chiasmata represent genetic cross-overs.

From Table 13 it may be seen that in Trillium and Melanoplus compensating and non-compensating pairs do not occur in equal proportions but that the former type are about twice as frequent.

Assuming that chiasmata represent genetic crossing-over, these data indicate that either non-random occurrence or recurrence takes place in these two diverse organisms.

In the Trillium preliminary data the average distance between chiasmata in thirty-five compensating pairs was 2.36μ and in fifteen non-compensating pairs was 3.27μ. Using a fourfold table, the association between length and type was found to be highly significant; $\chi^2 = 9.00$; P = less than .01. Since there is no terminalization the measurements made were probably close approximations to the original distances. In an organism such as Melanoplus which has terminalization, it is not possible to determine the original distances between chiasmata. Hearne and Huskins' figures show, however, that the average distance between chiasmata in thirty-nine pairs of the compensating type is 3.8μ and in twenty-six of the non-compensating type it is 4.6μ.

Table 13

Proportions of compensating and non-compensating chiasma pairs observed in Trillium and Melanoplus

	Compensating	Non-compensating	Total
Melanoplus (Hearne and Huskins 1935)	71	35	106
Trillium (preliminary data)	35	15	50
Trillium (main data)	244	147	391
	350	197	547

Note:- In Melanoplus it was possible to distinguish between compensating and non-compensating types in some cases where the exact type (a, b, c, etc.) of the configuration could not be determined.

(These were the numbers of the two types in which it was possible to trace the space relationships of the four strands completely.) Since distance between chiasmata is evidently associated with the type of the double exchange it follows that the two chromatids which cross-over at one level are not completely independent of those which have crossed-over at other levels. Thus, there is cytological evidence for non-random recurrence of crossing-over.

Lindegren and Lindegren (1937) have coincidently brought forward genetic evidence from Neurospora indicating that recurrence of crossing-over is not a random process. The ratios of two-, three-, and four-strand doubles obtained were 27 : 14 : 8. These data involved the attachment as a point of reference. It may be seen from Figure 10 that the double cross-overs involving only two of the four regions of chromosome have a much greater proportion of two-strand doubles than the double cross-overs involving three, or all four, of the regions studied.

It has been suggested that the apparent two-strand doubles involving regions adjacent to the spindle attachment could be accounted for by assuming a low percentage of asci with irregular nuclear distribution. It would seem, however, that this point could easily be checked, for if irregular nuclear distribution takes place after the second division it might also be expected after the third division, at which time its results would be quite obvious.

Lindegren and Lindegren (1939) have further data. In the chromosome studied, however, all the genes were on one side of the attachment, and the attachment itself was used as a marker. Under such conditions it would seem impossible to distinguish between two- and four-strand double cross-overs. It is possible, however, to distinguish between three-strand doubles and the two- and four-strand doubles combined. These, in cytological terms, give a

ratio of 24 : 17, compensating to non-compensating. In the previous Neurospora data the ratio was 35 : 14. Both these ratios deviate from the 1 : 1 ratio expected on a random basis, in the same direction as the cytological data from Trillium and Melanoplus, although the difference in the first is not statistically significant. It is not, of course, expected that such a deviation would be expected to be in the same direction in all materials; Beadle and Emerson's Drosophila data, for instance, show differences from Bonnier and Nordenskiold's, and both differ from Neurospora in the proportion of two-strand doubles.

Re-analysis of Beadle and Emerson's (1935) data has shown that their observations do not necessarily indicate that crossing-over is a random process as they assume. In fact, they seem to suggest, as do the Trillium data, that there is a direct correlation between the length of chromosome separating the two chiasmata and the proportion of two-strand and three-strand doubles (see Figure 11). The very small number of individuals in the critical classes, that is, the short double exchanges, must, however, be noted.

The data obtained by Bonnier and Nordenskiold (1937) give a striking indication of non-random crossing-over in Drosophila. They found a higher proportion of four-strand doubles than of three- and two-strand doubles, and that three-strand doubles are probably more frequent than two-strand doubles. Our analysis of these data indicates that they had an overwhelming excess of four-strand over two-strand doubles, there being 116 attached strands of a type that four-strand and three-strand doubles could contribute to and only nine of the type that could result from two-strand and three-strand doubles. An analysis of their data from the point of view suggested above is presented in Figure 12, in which it is shown that the two-strand

doubles tend to occur with greater frequency in shorter lengths of chromosome than in longer, and that the reverse is true for the four-strand doubles. It is therefore impossible to assume random recurrence of crossing-over in this strain. The correlation between length and type of double exchange is $n = -0.53$. By using a fourfold table and Yates correction, the value for χ^2 obtained was 8.43; P = considerably less than 0.01.

It has been noted by Bonnier and Nordenskiold that there are difficulties in the determinations of genotypic constitution of those individuals in which forked was used as a marker. If, however, the data from forked are disregarded there is still a significant association between length of chromosome region and frequency of two- and four-strand doubles. Incidentally, were the rigidity of the strands a major factor in causing both chiasma and chromatid interference in the way they suggest, it would be expected that the two-strand doubles would have a greater average length of interstitial region than the four-strand doubles, while on the contrary their data show that the interstitial regions of the two-strand doubles are all below average length. From these data it would seem quite certain that there are factors preventing complete randomness of crossing-over and chiasma formation.

It appears, however, that such interference must vary greatly in its expression. The Trillium data indicate that the length of the region of chromosome involved is one of the factors influencing the proportions of the different types of double exchanges. In this case a short length favors an excess of compensating pairs (two- and four-strand doubles) over non-compensating (three-strand doubles). Melanoplus data appear to be in agreement with this. There may be striking differences, however, in the actual proportions of compensating and non-compensating pairs in two Trillium corms having different chiasma frequencies.

Length of chromosome between cross-overs also appears to be a factor in the strains of Drosophila used by Beadle and Emerson (1935) and Bonnier and Nordenskiold (1937). In the former, short lengths of chromosome seem to be associated with an excess of two-strand doubles over three-strand doubles. In the latter short lengths of chromosome tend to occur between the points of crossing-over in the two-strand doubles and long lengths between those in the four-strand doubles. These strains differ greatly, however, in the actual proportions of the three different types.

In Neurospora the attachment appears to influence the type of chiasma pair including it. Thus there is a large excess of two-strand doubles symmetrically across the attachment and close to it, and a slightly smaller excess of two-strand doubles symmetrically across the attachment and farther from it.

There appears also to be a little evidence that undefined differences in a region of chromosome may influence the type of double exchange in that region. Thus, it may be seen in Figure 13 that the strain used by Beadle and Emerson appears to have a high proportion of two-strand doubles (relative to fours) in regions 20-30 and a low proportion in region 30-40.

It would also appear from this figure that such undefined differences might not be the same in any two strains. It is rather striking that the strain of Sturtevant differs from that of Beadle and Emerson in having exactly the opposite proportion of two-strand doubles in the regions mentioned.

One main generalization is strongly suggested by the genetic data. This is that two-strand doubles tend to occur more frequently in regions shorter than the mean length of all double exchanges, than in regions longer than the mean. This is similar to the observation recorded above for compensating chiasmata in Trillium and Melanoplus. Since compensating

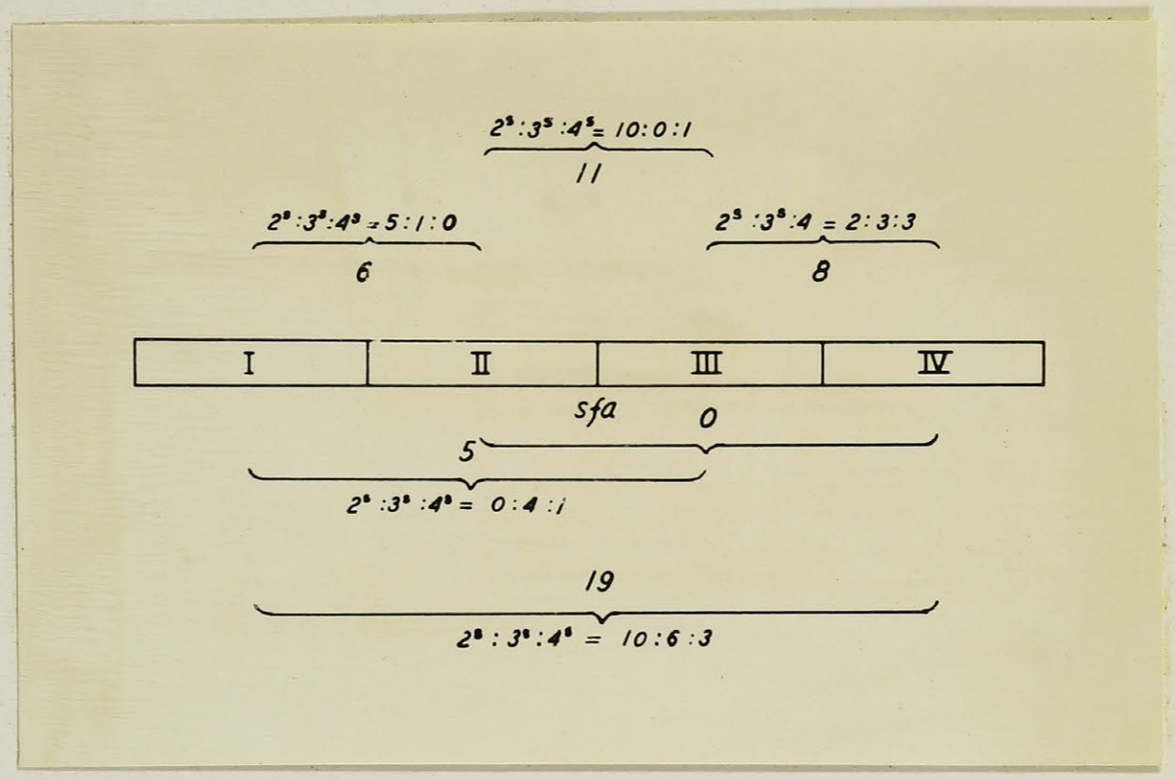

Figure 10. Numbers of two-, three-, and four-strand doubles observed in Neurospora, and their distribution with regard to the four chromosome regions involved. Redrawn from Lindegren and Lindegren (1937).

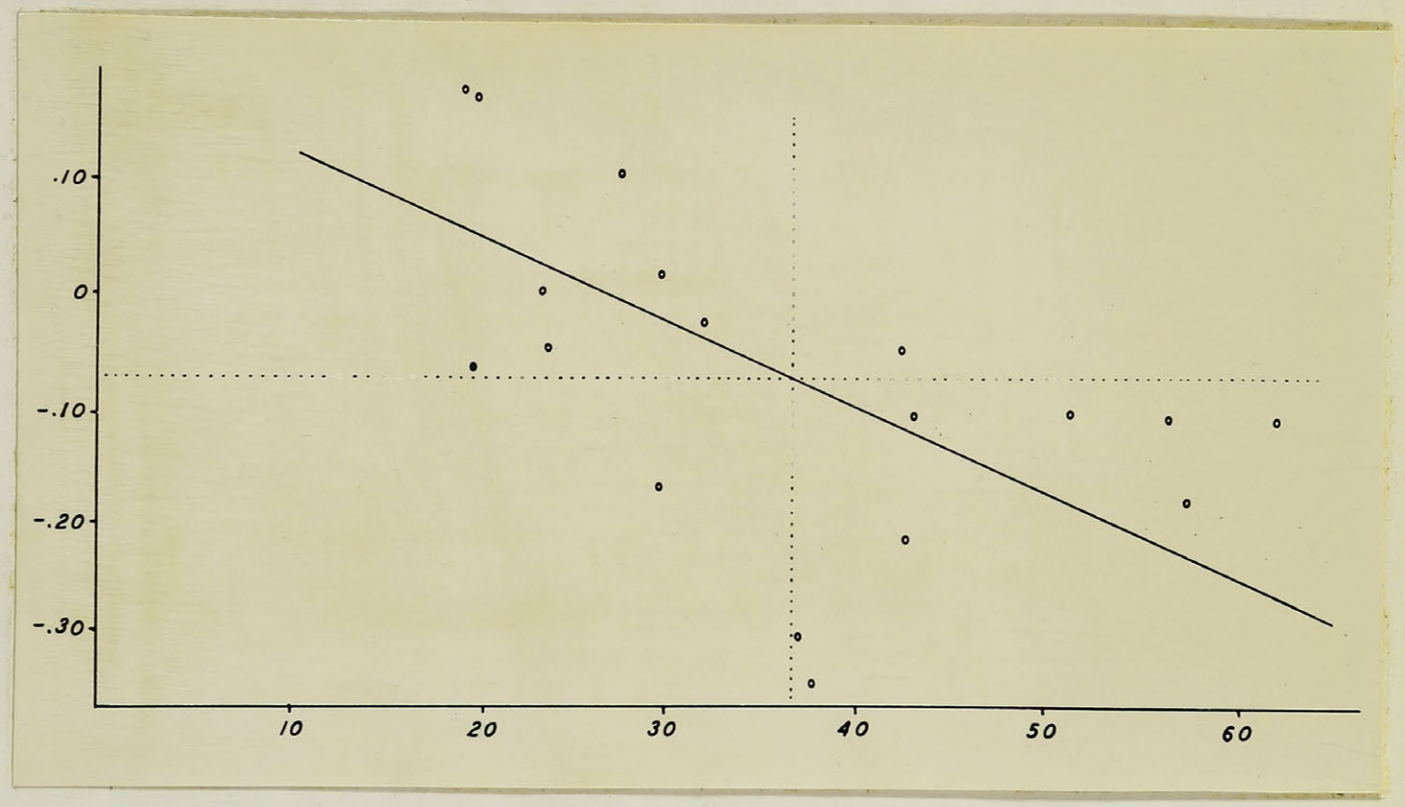

Figure 11. Excess of two-strand doubles in Drosophila over the number expected on a random basis (from the attached-X data of Beadle and Emerson, 1935, Table 6).

X axis: average distances between the two cross-overs in genetic map units.

Y axis: twice the proportion of two-strand doubles minus the proportion of three-strand doubles.

Solid line is the line of regression of the points. Dotted lines are averages of the values on the X and Y axis respectively.

The coefficient of correlation between X and Y = -.51; t = 2.39; P is between .05 and .02.

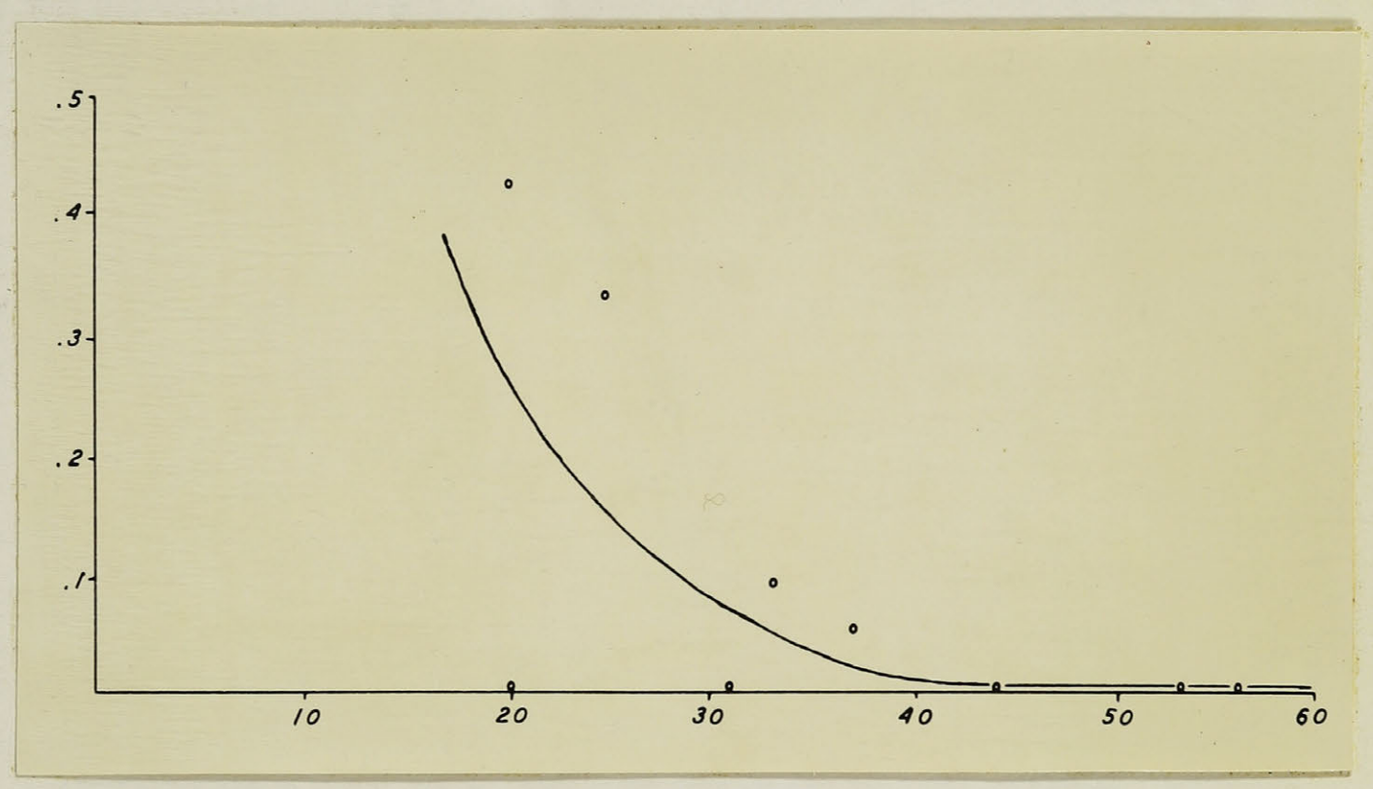

Figure 12. Proportion which two-strand doubles are of two- and four-strand doubles combined, per chromosome length in Drosophila (from the attached-X data of Bonnier and Nordenskiold, 1937).

X axis: average distance between the two cross-overs in genetic map units.

Y axis: the number of types $\frac{aaa}{bab}$ and $\frac{aba}{bbb}$ divided by the number of types $\frac{baa}{aab}$ and $\frac{bba}{abb}$ plus types $\frac{aaa}{bab}$ and $\frac{aba}{bbb}$.

The solid line was drawn arbitrarily to indicate the regression of Y on X.

Coefficient of curved linear correlation = 0.53.

Note: It is expected that three-strand doubles, if present, would contribute equally to each of these four types.

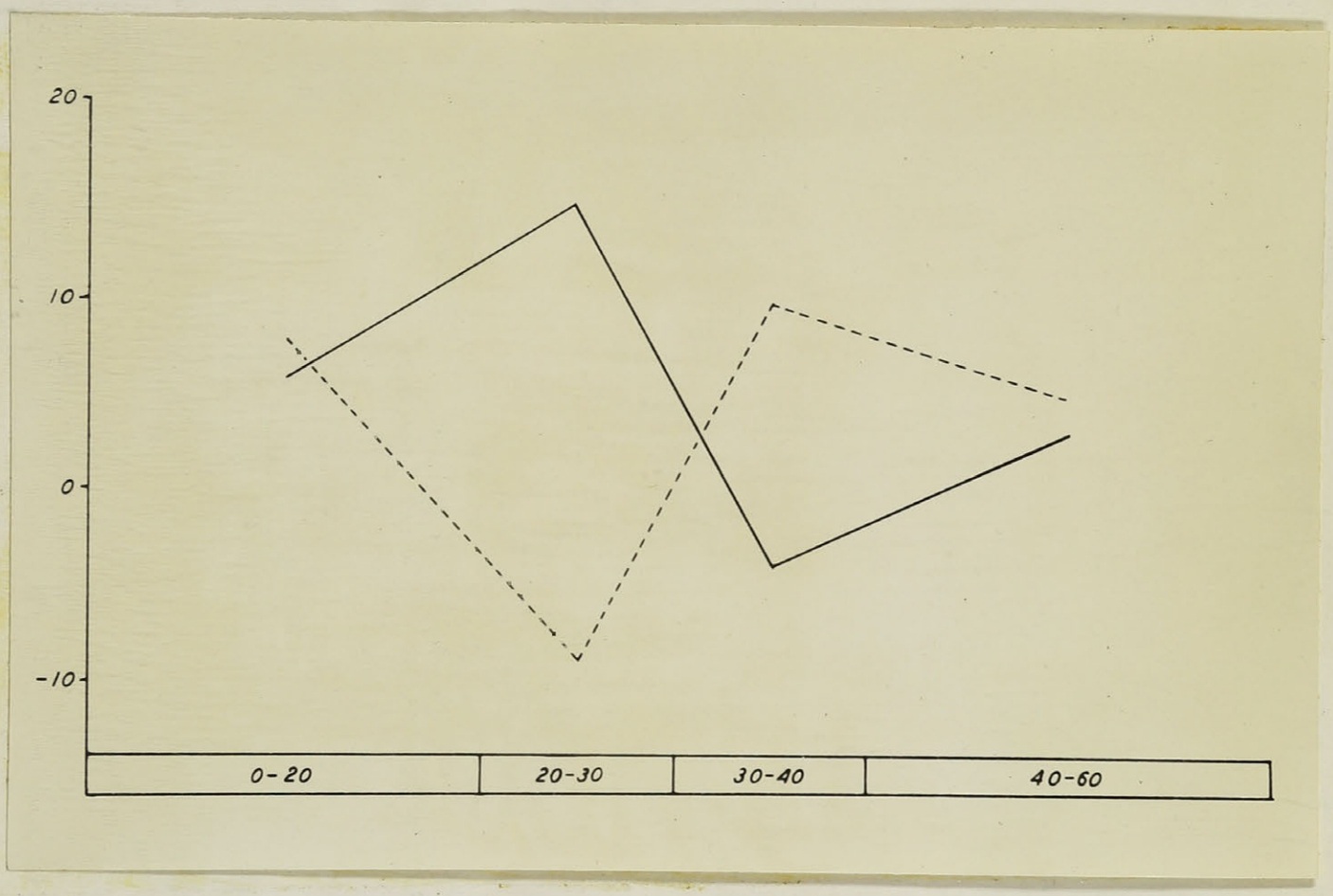

Figure 13. Excess of two-strand double exchanges over four-strand doubles with regard to the region in which they occur (from the attached-X data of Sturtevant, 1931, and Beadle and Emerson, 1935).

X axis: the region of chromosome, in map units, in which the center of the double exchange was located.

Y axis: the excess of two-strand double exchanges over four-strand doubles.

Solid line from the data of Beadle and Emerson. Broken line from the data of Sturtevant.

chiasmata may be either two-strand or four-strand double cross-overs, it is necessary only to assume that a considerable proportion of them are two-strand doubles in order to harmonize the genetic and cytological data.

(2) Probable Causes of Chromatid Interference

It has been pointed out previously that the more complex types of chiasma pairs observed indicate the presence of a certain amount of twisting between sister chromatids at the time of chiasma formation. It is also apparent that the greater the distance between two chiasmata the greater the probability of including chromatid twists in the interstitial region of that pair.

Neurospora data indicate that a strand having once become involved in a cross-over is more likely to be involved in the next if the distance between the two cross-overs is small, than if it is great. This may be termed negative chromatid interference. Such interference suggests the same thing that is indicated by the cytological data, namely, that prior to chiasma formation homologous strands which are in close contact at one level are less likely, because of chromatid twisting, to be those in closest contact at another level when the distance between the two levels is great, and more likely when the distance is short.

Thus, by assuming that at any level two homologous strands may be in closer contact than the other two, we have an extremely simple explanation both for negative chromatid interference and for the lengths of the different types of chiasma pairs observed.

It may be shown, however, that this explanation implies a tendency for adjacent chiasmata to be in opposite directions, in the pairs observed, particularly when the distance between them is short. Since this is in

itself of considerable importance, let us re-examine the types as illustrated in Figure 1.

It may be seen that the most complicated configurations are both the longest and the least frequent in their occurrence. This relationship does not necessarily hold for the simpler configurations since we are unable to determine the exact arrangement of the strands at the time of crossing-over. The degree of complexity (that is, the amount of twisting between sister chromatids) of the simpler types depends upon whether we assume that the two chiasmata were the same or opposite in their directions. The most frequent type (type a) is the simplest if we assume that the chiasmata were in opposite directions, but if we assume that the chiasmata were in the same direction it may be seen to have a half twist between chromatids in each of the two chromosomes, and is therefore not the simplest type.

If we assume, as Darlington does, that the two chiasmata of all types of pairs are in the same direction immediately following crossing-over, the configurations would at that time be similar to those represented in the lower row of Figure 1. These, although arranged in order of their frequencies of occurrence and their lengths, are not in order with respect to their complexity. If there is a consistent relationship between complexity, length, and frequency, we must assume that the two chiasmata tend to be in opposite directions, and that the arrangement of the strands at the time of crossing-over is more frequently like that represented by the first row of diagrams in Figure 1. Then, length, degree of complexity, and the reciprocal of frequency of occurrence, all vary together.

It has been suggested by Belling (1933) that overlaps of chromosomes may determine the positions at which chiasmata are formed. Assuming that two chromosomes overlap so that they touch in two places, the same chromatid

which touches at one place will have a greater chance of touching at the second if the distance between the two points of contact is small. This could explain the assumed absence of chromatid twists in the shortest type of chiasma pair, namely, type a.

It must be remembered that even if chiasmata are formed only at places of overlapping, it does not necessarily follow that the two chiasmata would have the same direction as the chromosomal overlaps. However, from a model it may be seen that breaking of the two touching strands and rejoining in the most direct manner results in chiasmata opposite in direction because the non-breaking strands remain as the crossing strands. This is illustrated in Figure 14. If such is the actual mechanism involved it would be expected that free (type a) configurations would be formed when there were no chromatid twists between the chiasmata. A half twist between two of the sister chromatids would result in a continuous (type b) configuration. A half twist in each of the two chromosomes would give a chromatid lock (type c) if the twists were in the same direction, and a chromosome lock (type d) if in opposite directions. Type e would result from two half twists in one chromosome and none in the other. The other types would be formed when a greater number of twists are included between the two chiasmata.

Still further implications of the suggested mechanism must be considered. If the chromosomes are twisted about one another instead of overlapped, then breaking and rejoining of the touching strands would, as Darlington has said, be expected to give rise to chiasmata in the same direction. It is possible on the suggested mechanism to estimate from the data the proportion of chiasma pairs derived from chromosome twists. The simplest type which could result from chromosome twists would be that in which there are no chromatid twists between the chiasmata at the time of formation. As may be seen from Figure 15, this will be chromatid lock (type c). Since, in

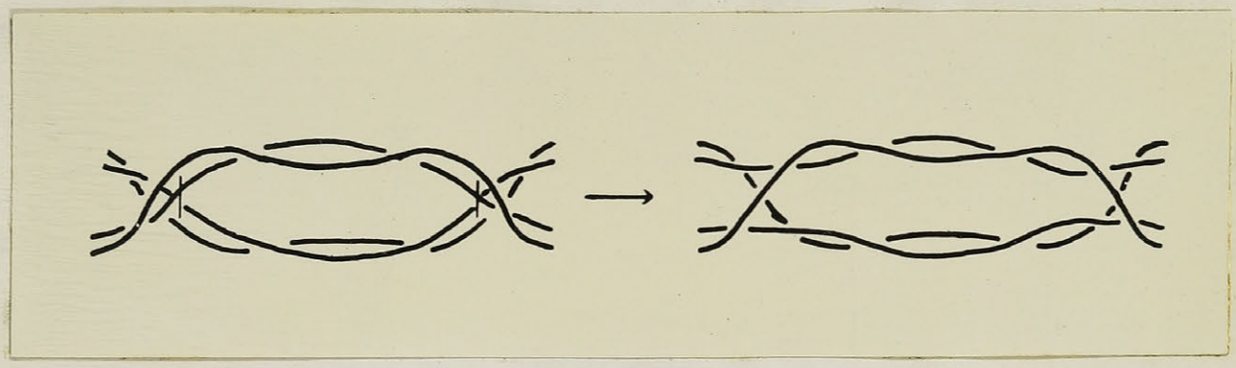

Figure 14. The simplest type of chiasma pair expected from chromosome overlaps if the touching strands break and rejoin in the most direct manner (type a).

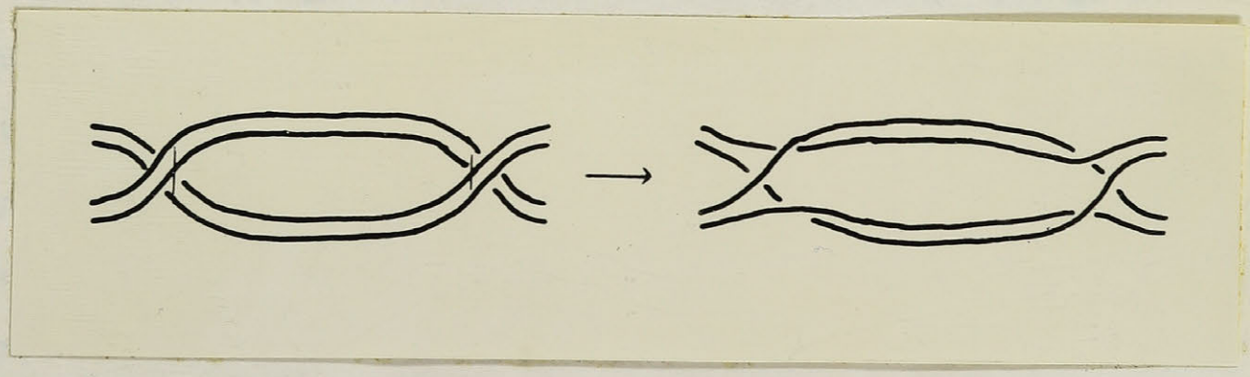

Figure 15. The simplest type of chiasma pair expected from chromosome twists if the touching strands break and rejoin in the most direct manner (type c).

lacking chromatid twists, they are like the overlaps assumed to produce type a, type c's which have arisen in this manner might be expected to be similar in length to type a, and to be the type most frequently produced when the chromosomes are twisted about one another.

Thus, assuming the theory to be valid so far, it is evident that type c could have arisen partly from overlapped chromosomes, each of the two having a half twist between chromatids, and partly from twisted chromosomes having no chromatid twists. Were it not for the latter class, types c and d would be expected to be equal in frequency and length, being derived from chromosomes having the same amount of chromatid twisting. The number of type c derived in the second manner would be expected to have two effects upon the type as a whole: (1) to increase the total frequency of type c above that of type d, and (2) to lower the average length.

The data can be interpreted as showing both these effects:

(1) Thus type c is practically twice as frequent as type d, the numbers observed being 44 and 20 respectively. Also, the average length of type c is less than that of type d, the two being 3.6μ and 4.7μ respectively. Since the numbers of types c and d derived from chromosome overlaps are expected to be equal, we may say that the excess of c over d represents the number of type c configurations resulting from twisting of chromosomes. If this is so, we have 24 of the type arising in the simplest manner (that is, without chromatid twists) from twisting of the chromosomes, and 250 of the type arising in the simplest manner from chromosome overlaps. Thus it is suggested that chiasma pairs resulting from chromosome overlaps are about ten times as frequent as those arising from chromosomes twisted about one another.

(2) With regard to the length of type c: it has been shown that on the theory it is probable that type c consists of roughly equal numbers of pairs derived from chromosome overlaps (these have a half twist of the chromatids in each chromosome and therefore a high average length) and pairs derived from chromosome twists (these have no half twists of the chromatids in each chromosome and therefore a low average length). The first group of type c would be expected to have an average length equal to that of type d (4.7μ), and the second group would have an average length equal to that of type a (2.5μ). Type c, being composed of equal numbers of these two groups, would be expected to have a mean length intermediate between 2.5 and 4.7μ. The mean length of type c in Trillium was 3.6μ, the exact mean of 2.5 and 4.7.

A further point regarding the average lengths of these configurations should be noted. We have assumed that one half of type c consists of pairs having a half twist of the chromatids in each chromosome, and one half of pairs having no chromatid twists. The average length of type c would supposedly be the same as that of a type having a half twist of the chromatids in one chromosome and no twists in the other chromosome. Such a type is b, which has an average length of 3.7μ in the main Trillium data; the average length of type c in the same material is 3.6μ.

The foregoing analysis does not in any way reveal the forces which result in breaking and rejoining of chromatids. All it shows is that from the observed configurations it seems probable that most chiasma pairs result when there is breaking and rejoining of strands at the points where overlapped or twisted chromosomes are in contact, and that if this is actually the case about ninety per cent of the chiasma pairs studied arose from overlapping of the chromosomes, and only about ten per cent from chromosomes twisted about one another.

Negative chromatid interference, then, appears to depend on the absence of chromatid twists in relatively short lengths of chromosomes.

Two facts, however, seem difficult to interpret on the mechanism assumed. The first of these is the existence of a significantly lower coefficient of variation of length in type c than in the other types of chiasma pairs. The second is the presence of positive chromatid interference in one strain of Drosophila. Whether they constitute a real objection to the above suggestion, must be considered carefully. It is possible that they merely indicate the action of other forces as yet unknown. The coefficients of variability have been calculated for each of the first four types (a, b, c, and d) and are given in Table 14. The differences in variability between types, and their degrees of significance, are given in Table 15. Type d has a coefficient of variability significantly greater than that of any of the other three types, and type c is significantly less variable in length than any of the other types. Type c, being a composite of two types having different average lengths, would be expected to show greater variability in length than the others. A partial explanation of this apparent discrepancy may be seen on examining Figure 5 which shows the frequency distribution of the different types in size classes. All except c have higher coefficients of variability due to the individuals in the large size classes. If we disregard the size classes above six microns the distributions are as would be expected on the hypothesis.

Let us consider the distribtuions within the size groups up to six microns. Although the numbers are small, there is a rather striking agreement with the hypothesis. Type c shows indications of a bimodal distribution. As would be expected, there is a peak in the same size class as the peak of type a. Another peak would be expected and does occur in a

Table 14

Coefficients of variability in length of the different types of chiasma pairs, and standard errors.

		n	v	σ_v
type a	(free)	190	3.640	.184
type b	(continuous)	144	3.241	.193
type c	(chromatid lock)	34	2.411	.293
type d	(chromosome lock)	16	7.437	1.340

Note:- n is the number of observations, v is the coefficient of variability, and σ_v is standard error of the coefficient of variability.

Table 15

Significance of the differences in variability between the types of chiasma pairs.

types	v_1	v_2	difference	$\sigma_{\bar{d}}$	x	P
a - b	3.640	3.241	0.399	0.266	1.50	.13
a - c	3.640	2.411	1.129	0.347	3.25	.001
a - d	3.640	7.437	3.797	1.388	2.73	.01
b - c	3.241	2.411	0.830	0.351	2.36	.02
b - d	3.241	7.437	3.196	1.350	2.37	.02
c - d	2.411	7.437	4.926	1.372	3.57	.001

Note:- v_1 and v_2 are the coefficients of variability of the two types respectively; $\sigma_{\bar{d}}$ = standard error of the difference; x = the difference of coefficients of variability divided by $\sigma_{\bar{d}}$; and P, the probability that the difference arose by chance.
(Type d is significantly more variable than any other type, type c is significantly less variable than types a, b, and d)

size class slightly above that in which the peak for type b occurs, that is, in the vicinity of the four micron class. Type d, although represented by only a very few individuals, has a peak above the first peak of type c, roughly in the position expected.

There seems to be no obvious reason for the unexpected number of individuals of types a, b, and d, in the high size classes, and since the numbers are too few to test any of the possibilities which could be mentioned, speculation would seem to be futile at the present time. The important thing shown by Figure 5 is that the distributions in those size classes which are well represented are in agreement with the hypothesis advanced.

The second possible difficulty is the extremely high frequency of four-strand doubles observed genetically by Bonnier and Nordenskiold. In paired chromosomes the chromatids touching at one point are more likely to be the ones touching at another when the distance between the two points is short. It would therefore be expected that a short distance between two chiasmata would be more favorable to the production of two-strand doubles than longer distances. Acting alone, such a factor would produce an excess of two-strand doubles when distances are short, and random proportions of the three types when the distances are sufficiently great. It must not be assumed, however, that this factor acts alone, and obviously in the data of Bonnier and Nordenskiold it does not. In order to explain the cytological observations it was assumed that chromosomes paired in such a way that two of the homologous chromatids were likely to be much closer at any one level than the remaining two. If pairing is closer between sister chromatids than between homologous chromosomes, this is the situation which would result. Actual observations of Trillium prophase (Huskins and Smith, 1935) suggest very strongly that such a situation exists in this organism. Belling (1928) has also reported a much more prominent primary split than secondary. It has

also been shown to be the situation offering the simplest explanation for the cytological data, and the genetic data of Beadle and Emerson. However, it practically excludes the possibility of a great excess of four-strand doubles, since when two homologous strands are in contact at one level, the remaining two are less likely to be brought into contact at an adjoining level than one of the remaining two and its homologue which was in contact at the first level. In fact, the probability of this latter being the case is just twice as great as that of the former. In order to explain an excess of four-strand doubles along the general lines of the present hypothesis it is necessary to assume that a tighter pairing exists between chromosomes, such that both pairs of homologous strands are in close contact at any point of overlapping. This would allow crossing-over between two homologous strands at one level and the remaining two at the next level, provided chromatid twisting is infrequent. Of the actual force producing an excess of four-strand doubles the only things which may be said are, that the above situation is necessary in order for it to act, and that conceivably it is similar to, or identical with, the force causing chiasma interference.

In this hypothetical situation the proportion of two-strand doubles would depend upon incompleteness of the pairing between both pairs of homologous chromatids, four-strand doubles upon completeness of such pairing and the presence of a positive force of interference, and three-strand doubles upon the amount of chromatid twisting and incompleteness of the above type of pairing.

An interaction of all these factors would provide a relatively simple explanation for the apparent anomaly of the strain used by Bonnier and Nordenskiold. It is of interest to note that the strain they used had higher crossing-over percentages than the standard. The cv - ct region was the only

exception to this. Beadle and Emerson's strain had lower cross-over values than the standard excepting in regions v - ct and ct - cv which had slightly more crossing-over than the standard. It is an intriguing possibility that the higher cross-over values of the former strain and the excess of four-strand doubles are both due to a tighter pairing of the chromosomes.

(3) Coincidence

It has been commonly assumed that the coefficient of coincidence, as measured genetically, represents chiasma coincidence. If, however, there is chromatid interference this will cause a difference between chiasma coincidence and genetic coincidence. The coefficient of coincidence is calculated from genetic data using the following formula:

$$\text{Coefficient of coincidence} = \frac{x\ n}{a\ b}$$

where x = the observed number of double cross-overs, n = the total number of observations (individual plants or animals), and a and b = the number of single cross-overs in the two regions concerned, regions A and B respectively. Coincidence as measured thus is, however, the product of both chiasma coincidence and strand coincidence if the latter occurs. The former is the number of chiasma pairs observed divided by the number of pairs expected in the absence of interference, and could be calculated from the above formula where x = the number of double chiasmata involving the two regions, n = the total number of cells observed, and a and b the numbers of single chiasmata in regions A and B respectively. Strand coincidence may be

defined as the proportion of double cross-over strands involved in two adjacent chiasmata divided by the number expected in the absence of chromatid interference. It could likewise be calculated from the above formula if it were possible to determine the positions of cross-overs on all four strands of a bivalent. In this case x = the number of double cross-over strands, n = the total number of strands observed, and a and b = the number of cross-overs at A and B respectively, which are part of a chiasma pair. This formula for strand coincidence may be expressed in terms of the numbers of observed two-strand and three-strand doubles. Thus x (the number of double cross-over strands) is equal to twice the number of two-strand double exchanges plus the number of three-strand doubles; n (the total number of strands) is equal to four times the number of chiasma pairs; a and b will each be equal to twice the number of chiasma pairs. The formula will now read:

$$\text{strand coincidence} = \frac{(2 \times \text{no. of two's} + \text{no. of threes}) \times 4 \times \text{total no. Xma. prs.}}{(2 \times \text{total no. of Xma. prs.})^2}$$

This is simplified to:

$$\text{strand coincidence} = \frac{2 \times \text{two-strand doubles} + \text{three-strand doubles}}{\text{number of Xta. prs.}}$$

It is known that chiasma coincidence varies upward from zero, and it has been shown here that it may sometimes exceed unity. The limits of strand coincidence are not known. The genetic evidence suggests that strand coincidence is greatest when the distance between the points of crossing-over is least. It is not possible to distinguish two-strand doubles from four-strand doubles cytologically, but if some of the assumptions made earlier are accepted for the moment we may estimate strand coincidence from cytological data:

We have assumed that type a consists almost entirely of two-strand doubles. Type b must on any assumptions, represent three-strand doubles. Type d, having a half twist of the chromatids in each chromosome, would be a four-strand double. Type c would, for reasons mentioned earlier in the paper, be half four-strand doubles and half two-strand doubles. From Table 2 it may be seen that, neglecting the few more complicated types, the ratio of two- : three- : four-strand doubles would be 212 : 144 : 42 in Trillium. From these and the formula given strand coincidence may be calculated for Trillium. Obviously this value would be greatest when calculated from the chiasma pairs having relatively short lengths. Conversely, it would be smaller if those chiasma pairs having relatively long interstitial regions are used in the calculations.

Although the assumptions on which these calculations are based are in almost perfect harmony with data derived from a wide variety of sources, they can be of use only as a possible check for the hypotheses.

One point may be checked now. If the hypothesis regarding the origins of the different types is correct, it would be expected that strand coincidence decrease regularly throughout the successive size classes in Trillium. Also, on the assumptions made regarding pairing in this material, it is highly probable that the values of strand coincidence would approach unity when the distance between chiasmata became sufficiently great. These have been calculated for each of the size classes and may be seen in Table 16. There is a decrease from 1.82 in the lowest size class to approximately unity in the highest. The decrease, although not exactly uniform, is as regular as could be expected, and the values obtained for the highest classes are very close to unity. In fact, if the data from the two highest classes are combined (these are poorly represented) the coincidence value obtained will be exactly one.

Table 16

Total numbers of the four major types of chiasma pairs in each of the size classes, and the coefficient of strand coincidence for each class, calculated on the assumption that type a are two-strand doubles, type b three-strand doubles, type c equal numbers of two- and four-strand doubles, and type d four-strand doubles.

size class	numbers of different types					coeff. of stand coincidence
	a	b	c	d	total	
0-1 microns	47	7	2	1	57	1.82
1-2 microns	63	30	9	2	104	1.57
2-3 microns	31	39	6	4	80	1.34
3-4 microns	22	29	7	1	59	1.37
4-5 microns	18	19	7	2	46	1.37
5-6 microns	6	7	3	1	17	1.35
6-7 microns	1	5	-	2	8	0.88
7-8 microns	2	4	1	2	9	1.11

1.00

Note:- The formula used was

$$\text{coeff. of strand coinc.} = \frac{\text{twice the number of 2's + the number of 3's}}{\text{the total number of chiasma pairs}}$$

where 2's are the two-strand doubles, and 3's are the three-strand doubles.

One further point should be mentioned. It is obvious that estimates of the frequency of chromatid twists may be made from these data. Should it be possible to vary the amount of chromatid coiling by environmental treatments during pre-meiotic mitosis, a means would be provided for testing the hypothesis advanced.

Various workers (Graubard 1934, Stephens 1936, and Mather 1936) have reached the conclusion that interference is absent across the attachment. The present author has been unable, however, to find in the literature an explanation for the coincidence values greater than unity reported across this region. While discussing the factors contributing to coincidence it might be well to point out that coincidence, as calculated, is not only a function of interference, but also of variability in cross-over frequency. In the absence of interference variability may result in coincidence values greater than unity. Thus, the values reported in Drosophila (Morgan, Bridges, and Sturtevant 1925) and in Primula (Gregory,de Winton, and Bateson 1923) do not necessarily indicate some kind of negative interference.

In order to demonstrate this let us take a hypothetical case in which coincidence is calculated from two adjacent regions. In each of these crossing-over may vary between ten and twenty per cent. We will assume that there is no interference between them. If a particular lot of flies has <u>either</u> ten <u>or</u> twenty per cent crossing-over in each of the two regions, the calculated coincidence will be unity. If, however, equal numbers of flies are used from two lots, one having ten and the other twenty per cent crossing-over in each of the regions, it is a matter of simple calculation to show that the coincidence value obtained from the combined lots will be 1.11. This illustrates the effect which variability may have on the coefficient of

coincidence. Since crossing-over near the attachment is subject to variability in Drosophila, it is not surprising that coincidence values as high as 1.3 have been reported.

It has been stated that an increase in chiasma frequency is accompanied by increased coincidence within the chromosome arms of both Trillium and Drosophila. This could be the result of an increased variability of cross-over frequency with interference remaining constant or a decrease in interference without change in variability, or of a change in both factors. Only when the regions in question are separated by sufficiently great lengths of chromosome, or by the attachment, will the effect of interference become negligible (absence of interference across the attachment is indicated by genetic data from Drosophila).

Trillium chiasma data from widely separated regions indicate that coincidence in the high chiasma frequency slides (1 and 2) is much greater than in the low chiasma frequency slides (3 and 4). This suggests that variability is also greater in the former material. The following explanation might be suggested: When chiasma frequencies are very low, variability must be restricted by the fact that large variations can only occur in an upward direction. Similarly, if chiasma frequency is assumed to be almost as great as interference will allow, large variations can only occur in a downward direction. It would seem that chiasma frequencies intermediate between these two possible extremes would allow the greatest opportunity for variation, since differences could occur freely in either direction.

This may be a satisfactory explanation for the coincidence phenomena in Trillium. The relevant facts are: (1) The highest coincidence values in our materials were obtained from widely separated regions in the slides having a

high chiasma frequency (the frequency in this case would appear to be intermediate between the two possible extremes). (2) Coincidence was less than this across the attachments (here the chiasma frequency is approaching the upper limit), and between distant regions within the arms in the low chiasma frequency slides (here the frequency is approaching the lower limit). (3) The increase in chiasma frequency which does occur near the attachment in slides 1 and 2 is accompanied by a reduction in coincidence across this region, supposedly because in these slides the frequency is brought still nearer to the upper limit.

Drosophila differs from Trillium in having more frequent crossing-over distal to the attachment than proximal to it. The data from the two organisms could be harmonized to some extent by assuming that in Drosophila, frequency of crossing-over distal to the attachment is near the upper limit, and that it is intermediate in the regions proximal to the attachment. On this basis two of the observed phenomena would be expected: (1) When an increase in crossing-over occurs it would be largely confined to the region of the attachment. (2) In material having such an increase, coincidence would be reduced across the attachment since the frequency in this region has been moved nearer to the upper limit.

Such an explanation obviously cannot be complete since it does not take into account possible changes in the factor or factors causing interference. In all probability such changes do occur. The observed increase in coincidence within the arms, accompanying an increase in cross-over frequency in Drosophila, would not be expected on the above assumption unless there were also a reduction in the intensity of interference. Change in variability may or may not occur in these regions. In the arms of Trillium

it is probable that both forces are acting together, although from Table 11 interference would appear to be negligible between two regions separated by four microns or more.

The evidence indicates that factors causing changes in chiasma frequency may change both interference and variability. Increase in crossing-over within an arm would appear to be associated to some extent with a reduction in interference, and it seems plausible that variability would to some extent be dependent upon both intensity of interference and frequency of chiasma formation.

From the data discussed in this section, it may be said that "coincidence" as ordinarily calculated is a function of chiasma interference, chromatid interference, and variability in crossing-over.

The following summary suggests in detail the factors which may influence these three components of coincidence:

- coefficient of coincidence
  - chiasma interference
    - the distance between chiasmata
    - environmental factors
    - the particular region of chromosome
  - chromatid interference
    - the distance between chiasmata
    - amount of twisting between sister strands
    - closeness of pairing between homologous strands
    - a force of positive interference
  - variability of chiasma frequency
    - chiasma frequency
    - chiasma interference
    - the particular region of chromosome

V. SUMMARY

1. Preparations of the first meiotic metaphase of Trillium have been obtained in which chromatid structure is sufficiently clear to allow the space relationships of all four strands of a bivalent to be traced.

2. The frequencies and average lengths of the eight cytologically distinguishable types of chiasma pairs have been determined for forty-eight complete cells.

3. The frequencies of these types are not those which would be expected on any of the current theories of crossing-over.

4. The types occurring least frequently are those having the greatest number of chromatid twists between chiasmata. They also tend to have the greatest average lengths. This would indicate a certain amount of chromatid twisting in existence at the time of crossing-over.

5. High chiasma frequencies increase the proportion of types characterized by short interstitial lengths, rather than shorten the mean length of any given type.

6. Mean interstitial length of the types varies as the inverse of frequency. These both vary with complexity if it is assumed that the chiasmata in a pair tend to be of opposite directions. A possible implication of this is that chiasma pairs are frequently conditioned by the presence of chromosome overlaps rather than twists in short regions.

7. Chiasmata in Trillium occur most frequently near the attachment.

8. Chiasma frequency is apparently not proportional to chromosome length in all five bivalents of Trillium.

9. Coincidence of chiasma formation is greater than unity across the attachment. Coincidence within the arms varies as the distance between chiasmata, reaching a value considerably exceeding unity. An increase in chiasma

formation is accompanied by increased coincidence within the arms and decreased coincidence across the attachment. This phenomenon has been observed genetically in Drosophila.

10. Compensating pairs of chiasmata are about twice as frequent within chromosome arms as non-compensating. Corms having a high chiasma frequency have a higher proportion of compensating pairs than those having low chiasma frequency. Assuming that chiasmata represent genetic cross-overs, this must mean that the strands involved in one cross-over are not independent of those involved in an adjacent cross-over, i.e., chromatid interference occurs. Genetic evidence from Neurospora and one study of Drosophila support this view. A re-analysis of the Drosophila data considered to give contrary evidence shows that they may not be in disagreement. Interstitial length between cross-overs has not been considered in any previous analyses concerned with this problem.

11. The chiasma pairs involving the attachment showed an excess of the non-compensating type. The number of observations was too small to be certain that the proportion differed significantly from that found in the arms.

12. Genetic data and indirect cytological evidence indicate that in Drosophila, Neurospora and Trillium shorter interstitial length increases the chance that a strand will be involved in both of two adjacent chiasmata. It is suggested that this is because pairing of chromosomes is such as to bring only one chromatid of each homologue into contact at most levels. A strain of Drosophila observed to have a great excess of four-strand doubles must necessarily have pairing between all four chromatids at most levels.

13. It is tentatively suggested that chromatid interference (or strand coincidence) is a function of (a) the intimacy of chromosome pairing (i.e., whether between one or two chromatids of each of the homologous chromosomes), (b) the amount of twisting between sister chromatids, and (c) a positive interference force, possibly similar to or even identical with,

chiasma interference.

14. Coincidence, as measured genetically, is a function of (a) chiasma coincidence, (b) strand coincidence, and (c) variability of chiasma frequency.

VI. ACKNOWLEDGMENTS

I wish to thank Dr. C. L. Huskins who suggested the study here reported, and who has made many valuable criticisms during the course of the work. I should also like to express my appreciation to Dr. S. C. Reed for his advice on the statistical treatment of the data, and to Dr. S. G. Smith and Mr. G. B. Wilson for the use of some of their excellent preparations.

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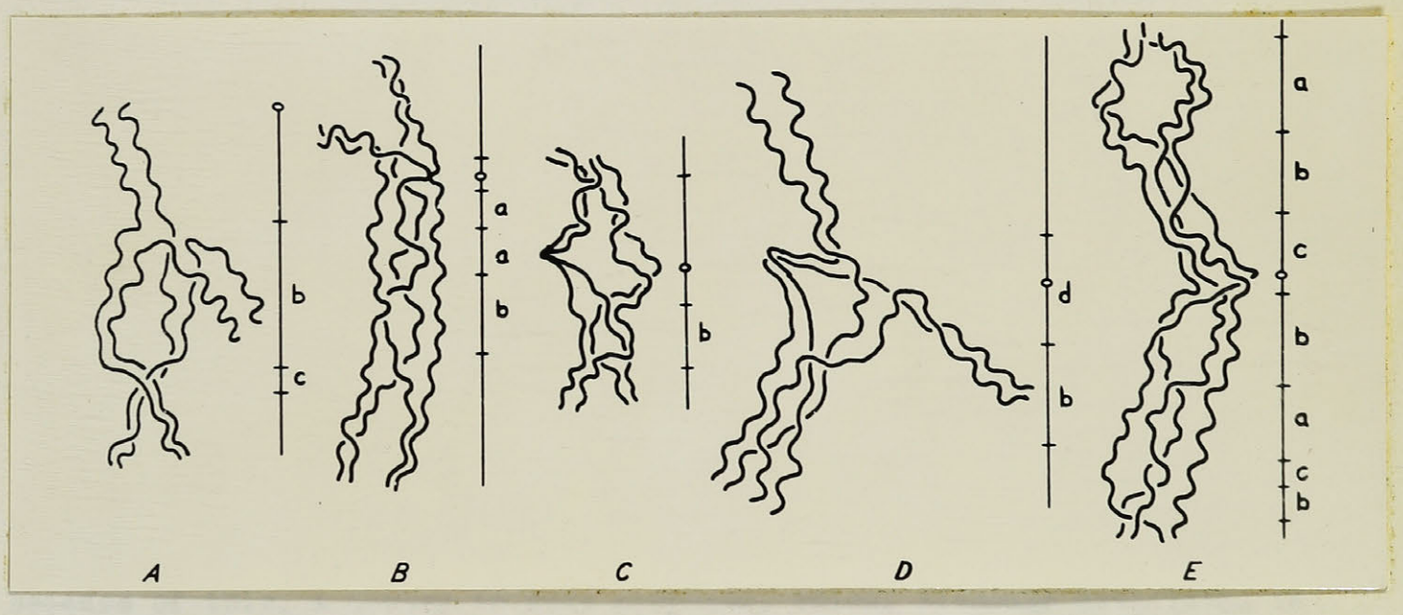
VIII. DESCRIPTION OF PLATE

Plate I.



Top row: A typical cell used in determining the space relationships of the four chromatids in the bivalents. Note the reduced coiling due to high temperature (approx. 20°C).

Middle row: Interpretation of nucleus in top row. The positions of the chiasmata are indicated on the lines beside each of the five bivalents. Small letters refer to the types of the chiasma pairs, illustrated diagrammatically in Figure 1. The position of the attachment is indicated on each of the lines by a small circle.

Bottom row: An example of extreme separation of the chromatids and reduction of the amount of coiling (from material grown at 25°C).

Note: The above photomicrographs are taken at intervals of 2 microns. Magnification approx. 800x

IX. APPENDIX I

A plausible mechanism of crossing-over

Introduction

From time to time theories have been advanced with regard to the mechanism of crossing-over. Although in most cases these were suggested by the observed nature of the chromosomes at pachytene, the probable time of this phenomenon, they have all involved certain fundamental assumptions for which there is no observational evidence. The value of such theories depends largely upon whether they can be tested.

Data gathered during the present investigation have rendered the acceptance of the previously suggested mechanisms extremely difficult. Because of this, a theory is here proposed, which, although artificial in some of its concepts, serves to unify a much greater variety of phenomena than has been possible before. Certain implications of the theory will be discussed at the end of the section, upon many of which it would seem quite possible to obtain experimental evidence.

As pointed out in the body of the paper, the observed types of chiasma pairs may be accounted for by assuming that breaks occur at intervals along the chromosomes frequently involving two adjacent strands at the same level, and that rejoining is in the most direct manner between homologous, or sister, ends. It is necessary to make one further assumption, namely that sister strands are tightly paired and that chromosomes are more loosely paired, lying more or less parallel, with overlaps and possibly a few twists occurring along their length. For some organisms this is practically a matter of observation (Belling on Lilium 1931 and 1933, Huskins and Smith

on Trillium 1935, and Hearne and Huskins on Melanoplus 1935).

Under such conditions breaks would occur at the same level in sister-strands, but since these are tightly paired and lying more or less parallel rejoining would be expected to occur in the original manner, and sister-strand crossing-over would be negligible.

It might also be pointed out here that translocations and inversions could readily occur if these assumed breaks happened where two chromosomes, or two parts of the same chromosome, were in close contact.

The theory is designed to explain (1) chiasma interference, (2) competition between bivalents, (3) the influence of the attachment on crossing-over, (4) the effect of environmental factors upon this influence, and (5) the absence of interference across the attachment.

The mechanism suggested

The basic assumptions are: (1) that the attachment and the chromomeres have electrical charges which may differ in intensity or sign, or both; (2) that breaking of the strands involves the establishment of electrical charges on the broken ends opposite in sign to those already on the chromomeres, and that it is to some extent conditioned by the presence and intensity of the charges of the proper sign in the surrounding medium.

The first of these is quite plausible if, as other authors have suggested, the attachment and chromomeres are amphoteric electrolytes, and if there is a change in pH during the division cycle. There is some indirect evidence suggesting that the cell is particularly acid during early meiotic prophase. Marshak (1938a and b) found that chromosomes were most easily broken by X-rays (supposedly through ionization) during the prophase stage. It was felt by Marshak that negative charges set in motion when the material was X-rayed broke the strands more easily at this stage because they had heavy positive

charges. Treatment with ammonia (supposedly raising the pH above the isoelectric point of the strands, and thus removing the positive charges) rendered the strands less susceptible to X-ray produced breaks. Incidentally, fewer cells were found to enter prophase after treatment with ammonia.

There is also some evidence indicating that the attachment is more acid (has a lower isoelectric point) than the rest of the chromosome. It has been shown by microchemical tests that the dark bands of salivary gland chromosomes are deposits of nucleic acid in regions the proteins of which are basic. (Caspersson 1936a and b, Hammarsten and Hammarsten 1935, and Schulman 1939). Since the attachment lacks these bands, we may assume that prior to the deposition of nucleic acid it was more acidic than the arms.

In order to explain the profound influence of the attachment upon crossing-over in the neighboring regions it is necessary to assume that it has a much greater area than an individual chromomere. The surface charge which it has will then be much more effective in influencing the frequency of breaks in that vicinity, than the charges on the chromomeres. The large size of the attachment is a matter of observation in many organisms. Whether or not there is a single minute "centromere" within this region is irrelevant to the present issue.

It may be mentioned here that Darlington has based an explanation of chromosome movement upon changes in pH and differences in the isoelectric points of the attachment and the rest of the chromosome. It will be shown later that these assumptions could also be used to explain the observed changes in chromonema length believed by Wilson and Huskins (1939) to be the immediate cause of meiotic coiling.

This mechanism of crossing-over differs from that suggested by Belling in that there is a force which will account for the phenomenon of interference.

It is unlike that of Darlington and of Sax in that no mechanical stress has been assumed.

Interference

On the suggested mechanism interference would be expected since the charges on the broken ends of chromosomes would repel similar charges from that vicinity; the scarcity of necessary charges in that region would decrease the chance of another break in nearby parts of the chromosome. It is also evident that breaks would interfere, not only with chiasmata formation in the same chromosome, but also with breaking in the regions of other chromosomes which happen to be in close proximity. Interference as the result of an electrical influence, would thus be a very simple explanation for the phenomenon of competition between bivalents.

Crossing-over near the attachment

We will go on to consider certain phenomena associated with the effect of the attachment upon crossing-over in the regions near to it. In some organisms it appears that the attachment increases the amount of crossing-over in its vicinity; in others the reverse is the case. Drosophila is an example of this latter type. Bridges (Morgan, Bridges, and Schultz 1937) has shown that the coefficient of crossing-over is less near the attachment region of the second and third chromosomes of Drosophila than distal to it. McClintock (unpublished) from cytological evidence has come to the conclusion that crossing-over in Zea Mays is least frequent in the region of the attachment. Beadle (1932) has shown that in a translocation involving chromosomes III and IV, the attachment region of the latter causes a decrease in crossing-over in the regions of chromosome III which are brought close to it. There is also evidence from studies of cytological maps (Beadle and others) indicating that low crossing-over is not primarily a property of the material of the chromosomes

but that it is conditioned by the distance from the attachment region.

A possible explanation of the above phenomena may be arrived at on the basis of the hypothesis assuming that the pH of the nucleus at the time of crossing-over is low, and probably near the isoelectric point of the attachment. If the pH were exactly at its isoelectric point the attachment would have neither a relatively positive nor a relatively negative charge, and would not influence crossing-over at all. Let us suppose that the pH is above this point. In such a case the attachment will have a negative charge. From our assumptions, the charge would tend to interfere with breaking and the establishment of other negative charges on the broken ends in the region of the chromosome adjacent to it. In this particular case the attachment will have decreased crossing-over. Drosophila and Zea are examples of this. The attachment region in such a case corresponds to Mather's description of it as a center of interference.

It might be mentioned here that examples of this influence are probably more common than has been supposed. Many organisms are known which have terminal or sub-terminal chiasmata at metaphase. These organisms have been said to have "complete terminalization"; it is equally possible, however, that they have relative localization of chiasma formation distal, rather than proximal, to the attachment. If the latter explanation is true it would be contrary to Mather's (1936) statement that, "when chiasma formation is localized it is nearly always confined to the spindle attachment region."

As the statement suggests, however, there are many organisms similar to Trillium in having a high frequency of chiasma formation near the attachment. Assuming that the alternative situation depends upon a pH above the isoelectric point of the attachment, high frequency of chiasma formation

near the attachment would be expected to result when the pH is below the isoelectric point of the attachment. Under such conditions the attachment would be positively charged. This would not be as intense as the charge on the chromomeres, but since the attachment is a relatively large body a charge on it will be more effective in attracting opposite charges from the surrounding medium. Thus breaking will be more frequent in its general vicinity than in any other part of the chromosomes. Trillium is an example of the many organisms having a high frequency chiasma formation near the attachment.

It should be mentioned here that the assumption that the attachment is a body of considerable size, and that its surface charge will therefore be of considerable magnitude even if of low intensity, is sufficient to explain the observed absence of interference across the attachment. The negative charge on the broken ends of chromosome one side of the attachment will have little or no effect upon the formation of similar breaks and the assumption of similar charges when a large charged body like the attachment occurs between the two points of breaking. This concept fits in with Mather's (1938) description of the attachment as an interference inhibitor.

Sensitivity of crossing-over to environmental factors in the region adjacent to the attachment would be expected if these in any way influence the pH at the time when crossing-over occurs. Factors tending to lower pH at this time would cause an increase in crossing-over; factors tending to increase the pH would cause a decrease in crossing-over. This effect would be largely confined to the region near the attachment. It is known that temperature, age, X-radiation, polyploidy, and the presence of inversions, all affect crossing-over in Drosophila. This is illustrated in Figure 1. A parallel cytological case of variation in chiasma formation is found in Allium (Emsweller and Jones 1935, Levan 1936, Maeda 1937). Gene differences

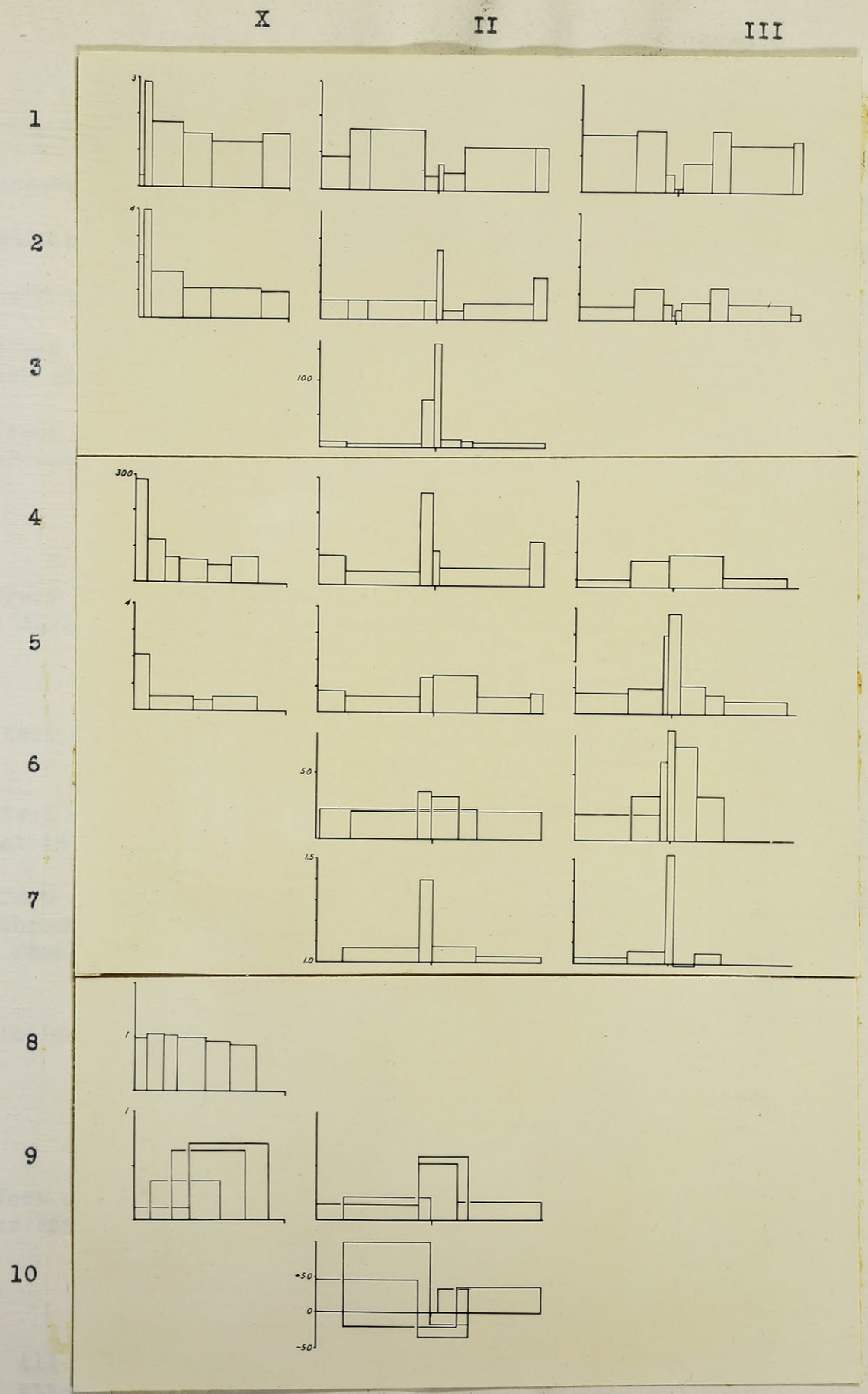


Figure 1. (Description on following page)

Description of Figure 1

Crossing-over in chromosomes X, II, and III of *Drosophila melanogaster*.

1. Coefficient of crossing-over. (Morgan, Bridges and Schultz 1937)

2. Coefficient of mutation. (Morgan, Bridges and Schultz 1937)

3. Effect of temperature in crossing-over. Per cent increase at 30°C over 25°C. (Graubard 1932)

4. Effect of inversions in two chromosomes on the normal chromosome. Per cent increase in crossing-over.
   (X, Steinberg 1936)
   (II, Morgan, Bridges and Schultz 1933)
   (III, Morgan, Bridges and Schultz 1932)

5. Effect of triploidy. Ratio of cross-over frequency in triploids to that in diploids.
   (X, Bridges and Anderson 1925)
   (II, Redfield 1932)
   (III, Redfield 1930)

6. Effect of age on crossing-over. Per cent decrease in ten day brood. (Bridges 1929)

7. Effect of X-rays. Ratio of cross-over frequency in treated material to that in the control. (Muller 1925)

8. Effect of the presence of the Y-chromosome on crossing-over in the X-chromosome. Ratio of cross-over frequency in XXY females to that in XX females. (Sturtevant and Beadle 1936, Bridges and Olbrycht 1926)

9. Coincidence between different regions.
   (X, Anderson and Rhoades 1930
   Bridges and Olbrycht 1926
   Anderson 1925
   II, Graubard 1932)

10. Effect of temperature upon coincidence. Per cent increase at 30°C over 25°C. (Graubard 1932)

Note:- All values are plotted against map distances. The position of the attachment is marked on each of the chromosomes.

(which on this theory could work through changing the pH at the time of crossing-over) are believed to be responsible for the presence of localization of chiasmata near the attachment, or the alternative in which they occur near the ends of the chromosomes.

The interpretation of the effect of inversions on crossing-over in normal chromosomes present in the cell is somewhat obscure on the basis of the hypothesis. It is far from an objection to the theory, however, since assumed failure of pairing in parts of the inverted chromosomes would cause an increase in the number of discrete strands at the time of crossing-over. The effect of this might be similar to the effect of triploidy. The latter has been observed to change the number and distribution of cross-overs within trivalents in much the same way that inversions affect crossing-over in the other or normal bivalents in the cell. More detailed suggestions regarding the mechanism could be made, for example, that the difference is due to the assumed positive charges being present on a greater number of strands. It would seem unprofitable, however, to work out such an explanation in detail at this stage.

Possible implications of the theory

It might be expected that chromosome breaking depends upon the chromomeres having a strong positive charge. From this it is not a very great step to the view that meiotic prophase differs from mitotic, at least partly, in that lower pH's exist in the former. If we assume that the meiotic prophase is more acid than the mitotic prophase it might be expected that the nucleus would show the characteristic phenomena of proteins at pH's considerably lower than their isoelectric point, namely hydration, swelling, and high viscosity.

Beasley (1938) has shown that the meiotic prophase nuclei in both plants and animals tend to be larger than the mitotic prophase nuclei (on the average about 3.25 times the volume).

More critical evidence has been found by Gustafsson (1939) who claims that in the facultative parthenogenetic plants, Antennaria, Hieracium and Eupatorium, sap intake of the nucleus determines whether or not it will undergo meiosis or mitosis. In sexual meiotic nuclei it is said that strongest growth takes place during early prophase and that intense hydration occurs mainly after prophase has started, in fact, about zygotene or early pachytene. The meiotic prophase nucleus increases about three or four times in size.

It is extremely interesting to note that the nucleus is increasing in size at the same time that the chromonema appears to be elongating (Belling 1931, Wilson unpublished), namely from leptotene to pachytene. The determinations of chromosome elongation are necessarily inaccurate at these stages as they must be estimated from measurements of distances between chromomeres. Although the measurements are not accurate beyond all doubt, they do constitute fairly conclusive evidence of elongation.

pH change is not necessarily the cause of these two phenomena but it is at least a very interesting implication both of these data and of the theory suggested in this paper.

This idea can be extended so that pH changes account for most of the observed phenomena associated with chromonema length, nuclear swelling, viscosity, chromosome movement, as well as the differences between meiosis and mitosis. Darlington has suggested such a mechanism for chromosome movements, but it has not been worked out in detail. It might be well to do this, using the available data on these factors, since certain expectations

on the basis of such a concept may be tested.

In Figure 2 is given an assumed pH cycle for both meiosis and mitosis, and the changes in chromonema length, viscosity, and nuclear size observed, or assumed on the theory. Solid lines represent observed changes and dotted lines assumed changes. The isoelectric point of the attachment is assumed to be the lowest, that of the chromomeres intermediate, and that of the poles the highest. The part of the spindle present on the metaphase plate probably has a high isoelectric point, not far from that of the poles.

During meiosis the following would be expected: First, the pH drops to the isoelectric point of the chromomeres. These, having no charge are therefore no longer repelling one another and a force (whose nature is not here considered) tending to cause them to pair, may now come into action. As the pH drops to approximately the isoelectric point of the attachment, the chromomeres develop strong positive charges. It is at this point that chiasma formation has been assumed. Because the pH has gone considerably below the isoelectric point of the chromonema this is at its maximum length. The following rise in pH will cause a shortening of the chromonema which becomes most extreme at its isoelectric point. A continuation of this change in pH will be expected to cause an increase in length, which, if taking place within a confined space, would result in coiling (Wilson and Huskins 1939). As the rise continues above this point both the arms and the attachments of the chromosomes develop a mutual repulsion.

If the poles are formed after the pH has risen above their isoelectric point they will repel one another and move to opposite ends of the cell. They will also be negatively charged and will repel the chromosomes and attachments forcing these to the metaphase plate. The pH now drops, and after passing the isoelectric point of the poles the latter becomes

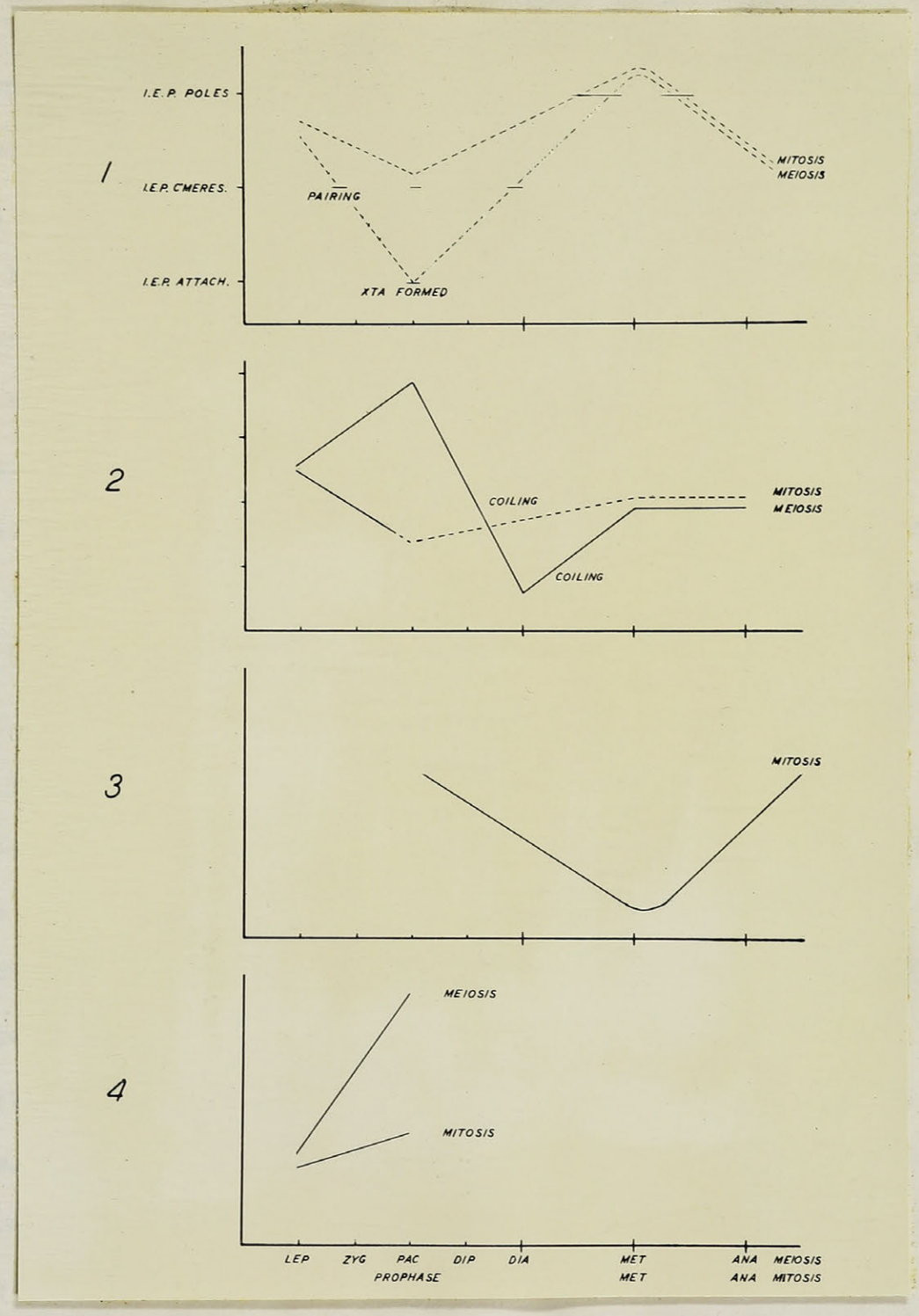


Figure 2. Observed and assumed variations in (1) pH, (2) chromonema length, (3) viscosity, and (4) nuclear volumn, during meiosis and mitosis. Solid lines represent observed changes, and broken lines represent changes assumed on the hypothesis. Assumed isoelectric points are given in the first graph for the poles, the chromomeres, and the attachments.

positively charged. The attachments which are negatively charged and which have been repelling each other right along are now attracted to the poles. Anaphase movement is initiated.

It might be expected that a fall in pH at anaphase would cause a decrease in the length of the chromonema. That this fails to occur is not a serious objection since the matrix around the strands might protect them from a sudden increase in acidity. Also the condensation of nucleic acid on the strands might readily result in a difference in their behavior at two different stages. A more serious difficulty is that the chromonema is not at its shortest when pairing takes place although pH has been assumed to be at the isoelectric point of the chromomeres. Discrepancies might occur, however, since it is not known that the isoelectric points of the chromomeres and of the connecting strands are the same.

For a number of reasons mitosis may be thought of as differing from meiosis in that it does not reach as low a pH during prophase. If the pH does not sink below the isoelectric point of the chromomeres these will continue to repel and will not have the opportunity to pair.

Assuming a normal isoelectric point for most of the proteins of the nucleus, meiotic prophase, because of its acidity, would be expected to be characterized by a greater degree of hydration, and a greater size of the nucleus. Further, this increase in size would be expected to take place between leptotene and pachytene.

One further piece of evidence indicates that if the pH is low during prophase it is probably high at metaphase. This is that viscosity appears to be low at metaphase (Chambers 1917, 1919, Seifriz 1920, Zimmerman 1923, Kostoff 1930, Kato 1933, Fry and Parks 1934). This is shown most clearly in the last of these papers, in which special precautions were taken to

determine the exact stage.

There are a number of expectations on the basis of this system which might with suitable material and methods be investigated experimentally. These are as follows:

(1) The difference in pH between different stages and between meiotic and mitotic prophase might be determined. Experimental difficulties at present prevent direct pH measurements by means of microinjection of indicators. It might be possible, however, to swell the mitotic prophase nuclei with dilute penetrating acids, to the size of the meiotic prophase nuclei; conversely by treating the latter with dilute penetrating bases their size might be reduced to that of the mitotic nuclei. Another method of approach would be to subject rapidly growing somatic tissue to treatments designed to decrease pH (acetic acid, CO_2, etc.) in the hope of causing divisions to some extent resembling meiosis. Similarly meiotic divisions might be altered by treating with chemicals which decrease acidity within the cell (ammonia and fat solvent anaesthetics which decrease respiration and CO_2 concentration within the cell). If asynapsis were produced by different agents which all tend to reduced acidity, there would be added reason to suspect that pairing depends upon reduction of pH to the point where the chromomeres are without charge.

(2) It has been suggested that viscosity at pachytene must be low in order to allow pairing. Actually the opposite might be expected on this theory since this is the time of maximum hydration and supposedly of lowest pH. Further, the viscosity in mitotic prophase nuclei would be expected to be less than in meiotic prophase nuclei. Since a number of fairly satisfactory methods are available for comparative determinations of viscosity it is quite possible that this may be tested.

(3) If localization of chiasmata near the attachment is determined by acidity and the occurrence of terminal chiasmata is determined by alkalinity of the nuclei at the time of crossing-over, it might be possible to produce such differences artificially. The pH of the cell is considered a very difficult thing to alter, however, chemicals are known which will produce such changes. The real difficulty is to produce very small changes without actually preventing cell division. It may be that the effect of these on the corresponding genetic phenomenon would be easier to investigate than the cytological one of chiasma distribution.

(4) If the isoelectric point of the attachments is lower than that of the chromomeres they would be expected on this reasoning to pair later than the chromomeres. Conceivably the ends of the chromosomes might behave like the attachment regions.

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X. APPENDIX II.

Variability of chiasma formation in Trillium

The data relevant to variability of chiasma formation in Trillium have been assembled in Table 1. Coefficients of variability are consistently lower for slides 1 and 2 due to the high chiasma frequency in these. The standard deviations of the number of chiasmata in an arm tend to be slightly less in slides 1 and 2 (less in six cases, greater in three cases). This difference may not be significant, but could readily result from the fact that chiasmata are more crowded in the chromosomes from these slides; this would tend to increase the interference between adjacent chiasmata and to reduce variability.

Standard deviations of the number of chiasmata per one micron region were obtained by grouping the regions from all the chromosomes. Variability, as expected from the coincidence values, is much greater in slides 1 and 2. This could be attributed to either or both of two causes: (1) an increase in the tendency of some regions, but not of others, to have chiasmata, and (2) an increase in the ease with which chiasma frequency may be changed in particular regions by undetected environmental differences. The former of these could increase coincidence when calculated as in this paper from data involving all the regions of all the chromosomes. Only the latter could increase coincidence between two genetically marked regions. There appears to be no way of determining to what extent this second force is acting in our material.

Table I

Variability of chiasma formation in high and low frequency material

	slides 1 and 2 (high Xma frequency)				slides 3 and 4 (low Xma frequency)			
	n	$\bar{m}$	σ	v	n	$\bar{m}$	σ	v
chiasmata per region*	74	12.07	5.23	41.1	74	5.07	3.56	70.2
chiasmata per arm								
A	24	2.67	0.94	35.2	24	2.00	0.82	40.8
Bl	24	0.96	0.61	63.8	24	0.42	0.69	172.2
Br	24	3.50	1.44	41.2	24	2.17	1.24	57.1
Cl	22	1.23	0.64	51.8	24	1.13	0.74	65.7
Cr	22	2.35	0.88	37.4	24	1.29	0.61	47.3
Dl	24	2.21	0.86	38.8	24	1.58	0.95	60.0
Dr	24	3.46	0.91	26.3	24	1.71	0.92	53.7
El	24	2.25	1.02	45.3	24	2.67	1.11	41.4
Er	24	3.42	0.99	28.9	24	2.71	1.20	44.3

n = number of regions or arms compared
$\bar{m}$ = mean number of chiasmata
σ = standard deviation
v = coefficient of variability

*The total chromosome complement is divided for this analysis into 74 regions each having a mean length approximating one micron. There is a slight error in these calculations due to the fact that regions on chromosome C of slides 1 and 2 and chromosome D of slides 3 and 4 are represented by 22 observations, and all others by 24. This would tend to produce a slight although apparently negligible increase in the standard deviation of both sets of data.

The per cent increase of chiasma frequency in the regions of the chromosomes from slides 1 and 2 over that in slides 3 and 4. has been measured. This is approximately the same for the regions adjacent to the attachment and for the regions in the rest of the arm, 42.4% and 39.0% respectively. This would suggest that the chiasma frequency of regions adjacent to the attachment has not reached an upper limit determined by interference. It could, however, be sufficiently near such a limit in slides 1 and 2 to reduce variability. There would seem to be no evidence on this point apart from the coincidence values across the attachment. Since we cannot be certain that there is no interference across the attachment this is not critical.