AN ANALYSIS OF CHIASMA PAIRS SHOWING CHROMATID INTERFERENCE IN TRILLIUM ERECTUM L.

C. L. HUSKINS AND H. B. NEWCOMBE[1]
McGill University, Montreal, Canada

Received September 19, 1940

INTRODUCTION

UNTIL comparatively recently there has been no compelling evidence that genetic "interference" involves more than the occurrence of one crossover reducing the chance of another occurring in nearby regions. Genetic data from Drosophila have generally been interpreted as indicating that there is no "chromatid interference," that is, that a crossover between two of the four chromatids does not influence which two are involved in the next. The occurrence of the first type of interference (now known as "chiasma interference") can be tested in any organism having a sufficient number of marker genes. Chromatid interference can be detected genetically in organisms such as certain fungi and mosses in which the four products of the meiotic divisions remain associated, thus making it possible to determine the genetic constitution of the four strands involved in two adjacent chiasmata, or in such abnormal stocks as "attached-X" Drosophila (and the X° or "closed X" arising from it) in which a permanent association at the right end of the two X chromosomes makes it possible to recover two of the four strands of a bivalent in the female progeny. The occurrence of more than 50 percent recombination, which has been reported in Pisum and Viola, would in itself indicate chromatid interference but the evidence in these cases requires further cytogenetic examination.

HEARNE and HUSKINS (1935) found definite cytological evidence of chromatid interference in chiasma formation in *Melanoplus femur-rubrum*. Recently, LINDEGREN and LINDEGREN (1937, 1939) have found genetic evidence of chromatid interference in crossing-over in *Neurospora crassa*. *Trillium erectum* has advantages over any other material yet studied for detailed cytological analysis of chromatid relationships and possible genetic interpretations, and a study of it was therefore initiated (HUSKINS et al. 1938) soon after chromatid interference was found in Melanoplus. Evidence is here presented showing that there are complexities in chiasma formation that have not previously been described or considered in analyses and theories of crossing-over.

[1] Facilities for this research were provided by a grant from the Rockefeller Foundation to McGill University. The preliminary studies were conducted while the junior author was an Empire Cotton Growing Corporation Scholar; the work was completed while he was a National Research Council of Canada Scholar.

In two adjacent crossovers there are three possible genetic types of crossover pairs or "double exchanges." These may be termed two-, three-, and four-strand doubles, since two, three and four strands respectively are involved in the two exchanges. If the chromatids are involved at random at the two crossovers these types will occur in the ratio of 1:2:1. A deviation from a 1:2:1 ratio of the three types of double exchanges would indicate that something is interfering with random association of chromatids in the two exchanges. If the two-strand double exchanges are in excess, strands involved once must tend to be involved again, and chromatid interference may be said to be negative. If four-strand double exchanges are in excess the reverse must be true, and chromatid interference may be said to be positive. A random proportion of these three types does not, however, necessarily indicate an absence of chromatid interference since there remains the possibility that both positive and negative interference are present at the same time, but that one or both are varying with position on the chromosome or the distance between crossovers. Under such circumstances the total effect might be a balance of the two.

With two- and four-strand double exchanges found in equal frequency there results a 1:2:1 ratio of non-, single- and double-crossover strands. Three-strand double exchanges give the three types of strands in the same ratios. Provided the numbers of two- and four-strand double exchanges remain equal an excess or deficiency of three-strand double exchanges will therefore not affect the ratio in which non-, single- and double-crossover strands occur. It will, however, indicate some form of chromatid interference. If all chiasmata represent genetic crossovers the three-strand double exchanges can be distinguished cytologically from the two- and four-strand doubles combined.

In cytological studies where the chromosomes are so fixed and stained that all four chromatids can be traced through successive chiasmata it is, of course, possible to study both chiasma and chromatid interference provided it is assumed that all, or at least an overwhelming proportion of all, chiasmata represent genetic crossovers. In many individual cases it now seems well established that chiasmata do result from crossing-over (BELLING 1929, DARLINGTON 1930; for reviews see DARLINGTON 1937, MATHER 1938); hence this is a reasonable working assumption which we herein adopt. The evidence for MATSUURA'S (1940) interpretation, which involves two mechanisms of chiasma formation, is not considered adequate. Pending more compelling evidence "*entia non sunt multiplicanda . . .*" The chief limitation to the cytological method is that the chiasmata when examined may not be in the positions in which they were formed; this is definitely the case in those organisms in which terminalization occurs. A second limitation is that it is impossible to determine whether the two

chromatids which cross each other at a chiasma are genetically the cross-over or non-crossover strands. Three causes are here involved: (a) We do not know which two of the four strands are involved at the time of chiasma formation. (b) After chiasma formation twisting of the chromosomes can obscure the original arrangement even if the sister strands remain closely associated and homologous pairs of strands are widely separated. (c) When the four chromatids are all more or less widely separated from each other, or if alternate internodes are at right angles, it is the angle from which a chiasma is viewed that determines which strands appear to cross.

Despite these limitations certain data important for an analysis of crossing-over can be obtained from purely cytological observations of favourable material. Some of these cannot be obtained from purely genetic studies which have their own serious limitations. For instance, they give us no idea of the chromatid arrangements producing the single, double and non-crossover strands. Obviously it would be better to have data from an organism which is favourable for both genetic analysis and cytological study at meiosis, but this has not yet been found.

Using observations of various authors on chromosomes not stained to show internal structure and therefore yielding only approximate data, HALDANE (1931) showed that the frequency of chiasma formation in a bivalent has a much smaller variance than would be expected if each is an independent event. This indicates the presence of "chiasma interference" which may be assumed to be the same as ordinary genetic interference in crossing-over. The amount of interference cannot, however, be determined accurately from such studies owing to the impossibility of determining the true chiasma frequency and distribution in "bulk-stained" chromosomes.

In *Melanoplus femur-rubrum* HEARNE and HUSKINS (1935) found 71 compensating pairs of chiasmata and 35 non-compensating (highly significant deviation from expected 1:1; $\chi^2 = 12.23$). In the compensating type the paired strands separated by one chiasma are reunited by the second. This may be caused (speaking descriptively and disregarding for the moment the question of which are the genetic crossover strands) either by the same two strands being crossed at both chiasmata (giving a "reciprocal compensating" pair) or by the alternative pair being crossed at the second one (giving a "complementary compensating" pair). In the absence of visible differences between homologues, however, we cannot know the relationship of the ends of the chromosomes after diplotene opening out, and any attempt to distinguish these two types of compensating chiasma pairs cannot be other than merely descriptive and superficial, even from the purely cytological point of view. Obviously then, in attempting a genetic interpretation, it cannot be determined whether they are two- or four-strand double crossovers. However, for the purpose of demonstrating

the existence of chromatid interference it is quite sufficient to show that compensating and non-compensating chiasma pairs do not occur with equal frequencies. The frequency of the former in Melanoplus being twice that of the non-compensating pairs indicates that there must be chromatid interference in chiasma formation but it does not tell whether this is positive, negative, or more complex in nature. Were there no chromatid interference they would be expected in equal numbers. The converse, however, does not necessarily hold, that is, equality of compensating and non-compensating chiasma pairs does not prove the absence of chromatid interference. For instance, two-, three-, and four-strand double exchanges occurring in the ratio of 3:4:1 and therefore producing double-, single- and non-crossover strands in a ratio of 10:12:10, would give equality of compensating and non-compensating chiasma pairs.

Trillium erectum appears to have little or no movement of chiasmata before anaphase (HUSKINS and SMITH 1935); its chromosomes are very large and few in number, and by appropriate methods the four chromatids of each bivalent may be clearly differentiated and sufficiently separated for them to be traced through all the chiasmata along their length—in some cases even through the attachment region. SAX (1936) pointed out that in HEARNE and HUSKINS' Melanoplus figures there were four cytologically distinguishable types of chiasma pairs. In Trillium eight distinct types have been observed. These were found to differ in average length as shown herein. This introduces a new factor to be considered in the genetic analysis of interference. A reanalysis has therefore been made of certain published Drosophila data, pending a new genetic study with stocks especially made up for the purpose. BEADLE and EMERSON (1935) from an analysis of crossing-over in an attached-X strain of Drosophila concluded that there was no evidence of chromatid interference. WEINSTEIN (1936) reached the same conclusion from analysis of many published and original data. BONNIER and NORDENSKIÖLD (1937) reported positive chromatid interference in *D. melanogaster*, but some of their evidence is disputable.

MATERIALS AND METHODS

A preliminary study was made in 1936–37 from one of the best of HUSKINS and SMITH'S (1935) slides. The results were reported in detail only verbally at the Summer Meeting of the Genetics Society of America (summary in HUSKINS et al. 1938). They are here presented separately from the main study which has been made during 1938–39. For the main study two slides made for WILSON and HUSKINS' (1939) analysis of chromosome coiling have been used in addition to a number made especially for present purposes. The preparation of the latter differs from that of the

first three slides only in the conditions under which the material was kept prior to smearing.

Pollen mother cells at early first metaphase were smeared on slides, desiccated for from 20 to 30 seconds and fixed overnight in La Cour's 2 BD. Following this they were bleached with hydrogen peroxide in 70 percent alcohol and stained in iodine-crystal violet according to the schedule of HUSKINS and SMITH (1935). In the special preparations the washing before and after bleaching was reduced to little more than a rinse.

In the first three preparations the spiralling of the chromonemata made it difficult to trace each of the strands throughout its length without at some level confusing one with its pairing partner. However, by raising the temperature at which the Trilliums were kept while undergoing meiosis the spiralling was greatly reduced. The material for WILSON and HUSKINS' preparations (slides 1 and 2) was kept in small tanks at 16°C. The two recent preparations analyzed in detail (slides 3 and 4) were made from material grown at room temperature, 18°–22°. The material used by HUSKINS and SMITH in the preliminary study was from corms kept at slightly lower room temperatures.

In the preliminary study, due to the coiling, chromatids could be traced only in some of the bivalents in a cell. In the main study camera lucida drawings at a magnification of 4100× were made of all the chromatids in 12 cells from each of four slides. A Zeiss 1.5 mm 1.3 N.A. objective and a 20× ocular were used.

OBSERVATIONS

Preliminary data

Only four types of chiasma pairs, the same as those found in Melanoplus, were discovered in the preliminary study. They are those designated a–d in figure 1, which shows diagrammatically all the eight types (a–h) later found. The frequencies of the four types, a–d, in the fifty pairs of chiasmata initially studied are 33:15:1:1 (table 1). The mean length between the chiasmata in the two most frequent types, a and b, is 2.3μ and 3.3μ respectively. A χ^2 test, using a four-fold table for the two types and the numbers longer and shorter than the mean gives a value of 6.7, P = .01. The difference is therefore significant. Type a is a pair of compensating chiasmata and type b is non-compensating.

Main data

In the 48 cells drawn from slides 1, 2, 3 and 4 there was a total of 1011 chiasmata. There were 508 adjacent pairs, not including those straddling the spindle attachment. In 391 of these pairs it was possible to trace with

a high degree of accuracy the positions of all 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.

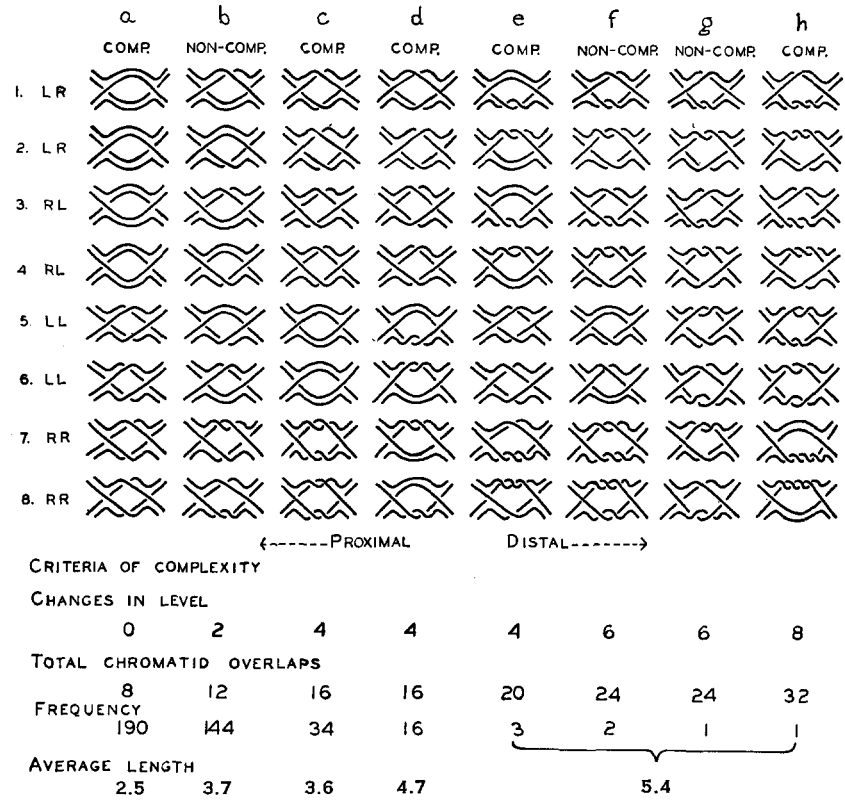

FIGURE 1.—Diagrammatic representation of types as used in analysis of chiasma pairs, see text.

Actual configurations of these four types are shown in figure 2 which is diagrammatic only in that the chromatids are depicted without coiling. In figure 3 the complete complement of one nucleus from slide 3 is illustrated. As mentioned above, slides 3 and 4 had reduced coiling. It will be noted that in three bivalents the chromatids can be traced through the attachment region of both homologues, though not with as great certainty as elsewhere along their length.

In interpreting these three dimensional configurations and illustrating them in one-plane diagrams, account must be taken of differences which may result from the angle of view and from the movement of chromosome ends in the "opening-out" which occurs after the time of crossing-over. A com-

plex configuration may present eight different aspects if angle of vision and possible movement of ends is taken into account. In figure 1 the eight distinct types are shown in the eight columns and the different aspects in the eight rows of each column. The derivation of the eight rows in figure 1

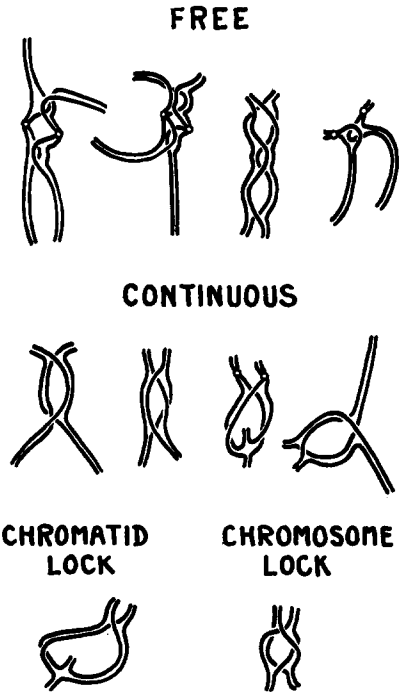

FIGURE 2.—The four commonest types of chiasma pairs drawn with chromonema coils omitted.

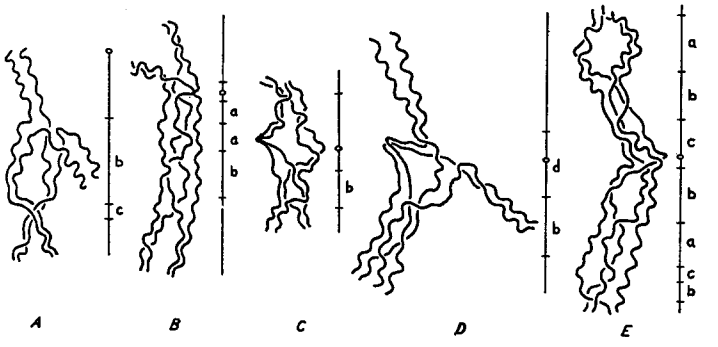

FIGURE 3.—The five bivalents in one nucleus of *Trillium erectum* slide 3. Note the four types of chiasma pairs and the split attachment regions.

which show diagrammatically the various aspects which each of the eight types of chiasma pairs may assume is as follows: Row 1 is the arbitrary standard representation of chiasma pairs with both ends of one homologue

pointing downwards; in row 3 both ends have been inverted. Row 5 has only the right-hand ends of the "standard" representations of row 1 inverted; row 7 has only the left ends inverted. Rows 2, 4, 6 and 8 are rows 1, 3, 5 and 7 viewed from behind but with the attachment still to the left, that is, the standard representations have been rotated through 180° on their longitudinal axes.

It will be seen from the figure that the effect of 180° rotation varies with the complexity of the configuration. Types a and c are unaffected. Rotation of types b, d, e, f, g and h gives even-numbered aspects distinct from their corresponding odd-numbered aspects.

In obtaining mirror images the mirror must be held above or below, not at the end, as that would reverse the "chiasma direction" relative to the attachment which is consistently at the left-hand side in this classification. Owing to the simplicity of type a, mirror images are indistinguishable. They are likewise indistinguishable in type d since mirror images of 1, 2, 5 and 6 are the same as the representations in rows 3, 4, 7 and 8 respectively. In type c, although 180° rotation gives even-numbered aspects identical with odd-numbered aspects, their mirror images are distinguishable. In all other types a mirror image of each aspect is distinguishable.

The joint effect of rotation and mirror imaging will therefore be to produce 16 distinct configurations of types b, e, f, g and h chiasma pairs. Types c and d will each comprise only eight but for different reasons; in type c four result from mirror imaging, in type d four result from rotation. Due to its complete symmetry type a consists of only four distinct configurations.

In table 2 are given the chiasma frequencies, the number of adjacent pairs, the number of these in which the strands could be traced, and the numbers of each of the different types. Table 1 gives the average lengths of each of the observed types in the preliminary work, the four Trillium preparations of the main study and the work of HEARNE and HUSKINS (1935) on Melanoplus. From the drawings of the latter it has been possible to measure the distance between chiasma pairs and to distinguish according to the present classification 65 of their 106 configurations.

Pairs of chiasmata, types and lengths

In table 1 the types of chiasma pairs are arranged in order of decreasing frequency, which, as we shall see, is essentially in accord with their order of increasing complexity. There are two possible criteria of complexity: (1) Each type may be drawn in the eight different ways possible (neglecting mirror images), that is, twisted so that the directions of the two chiasmata are LR, RL, LL, and RR, and the total numbers of chromatid overlaps then counted. For types a to h these are 8, 12, 16, 16, 20, 24, 24 and

32 respectively. (2) If at the ends of a chiasma pair two strands are thought of as being on a higher level and two on a lower level, then the number of changes in level of the four strands may be counted. The numbers of changes in level for any one of the eight possible representations of each type a to h are 0, 2, 4, 4, 4, 6, 6, and 8 respectively. By either criterion type c is equal in complexity to type d. The difference is that the second method would indicate that type e is also only of the same degree of complexity as types c and d.

Mirror images were disregarded in the original study. They have since been analyzed by DR. S. G. SMITH from the original drawings. It is found that there are 106 plain and 78 mirror images, a significant deviation from

TABLE 1

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			

equality—$\chi^2 = 4.26$, $P = .05 - .02$ in the six types b, c, e, f, g, and h in which they are distinguishable.

Complexity is clearly correlated with the frequency with which a particular type occurs, and not only do the simplest types of chiasma pairs occur must frequently but within the four common types the average length of the configuration (that is, the distance between two chiasmata) is least in that occurring most frequently, type a, and the greatest in that occurring least frequently, type d. Types b and c are intermediate. Types e, f, g, and h are represented by only very small numbers of observations and the individual lengths are not obviously correlated with frequency. The average length for the combined four groups is, however, greater than that of any of the more frequent and less complex types.

Similar data derived from HEARNE and HUSKINS' (1935) Melanoplus figures have been included in table 1. It was not expected that differences in length of different types would be as noticeable in Melanoplus chromo-

somes because in it the strands were in many cases very widely separated. Apart from terminalization, such a separation of the chromatids, by increasing parallax, makes determination of the positions of the chiasmata less definite and renders determinations of the interstitial distances less accurate when made from drawings that did not especially take this into account. It will increase variance and reduce the chance of discovering a statistically significant difference between the interstitial lengths of the types. Despite this, it was found in Melanoplus that type b configurations were longer than type a, just as they are in Trillium. The average length of type a was 3.5μ and of type b 4.8μ. A test of significance applied to these data gave a χ^2 value of 5.6, $P = <.02$.

In the main data the significance of differences in length has been tested in a number of ways. Considering the first two slides alone, it will be seen from table 2 that in each of the five bivalents from each slide, that is, in ten separate classes, type a is shorter than type b. The probability of this occurring by chance is one in 2^{10}, that is, $P = .001$. The same method when applied to the second two slides gave similar though 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 number of observations in some of the chromosomes was therefore necessarily small.

The χ^2 test was also used as with the other data (see table 3) to find the significance of the difference in mean length between types a and b. The data from slides 1 and 2 gave a χ^2 of 19.52; those of slides 3 and 4 a χ^2 of 1.25; $P = <.001$ and $<.30$ respectively.

The lack of significant difference in slides 3 and 4 requires further consideration. A reduction in chiasma frequency must have two effects (a) to increase the average distance between chiasmata and (b) to reduce the total number of observations. As may be seen from figure 4 the lower chiasma frequency slides 3 and 4 have a sharp reduction in the number of chiasma pairs in the shorter classes, and a slight increase in the number in the longer classes. From figures 5 and 6 it may be seen that type a is in great excess only in the shorter classes of slides 1 and 2. In the longer classes types a and b occur with nearly equal frequency. This must necessarily decrease the difference in their average lengths which together with the reduction in number of observations would be responsible for the low χ^2.

In order to determine the significance of the differences between the mean length of the types when compared with one another, the standard error of the mean was calculated for each. The standard errors, and the significance of the differences, 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, and type c than type d.

TABLE 2

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	2.2	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 and 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	3.4	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	3.7	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 and 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 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	χ^{2*}	P
	NUMBER	AV. L.	NUMBER	AV. L.	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	31	3.8	1.5	15.16	<.001
Slide 2	53	2.0	46	3.0	1.0	12.04	<.001
(1 and 2)	122	2.2	77	3.3	1.1	19.52	<.001
Slide 3	27	3.7	21	5.2	1.1	0.167	<.70
Slide 4	41	2.7	46	3.6	0.9	3.87	.05
(3 and 4)	68	3.1	67	4.1	1.0	1.246	<.30
(1, 2, 3 and 4)	190	2.5	144	3.7	1.2	15.87	<.001

*χ^2 was calculated by means of a 2×2 table using the numbers of types a and b longer and shorter than their joint mean.

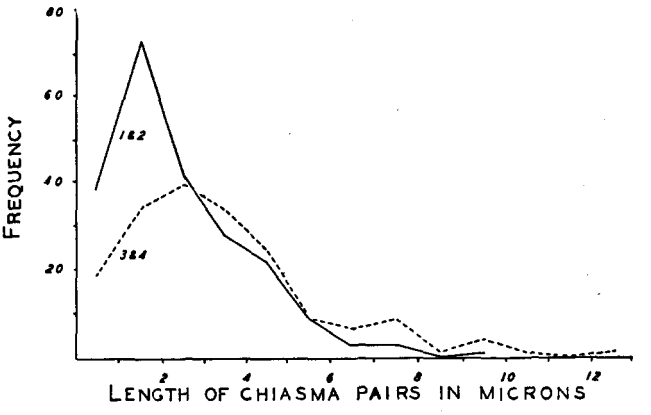

FIGURE 4.—Frequency distribution of chiasma pairs of different lengths (from table 5). Solid line—slides 1 and 2 (high chiasma frequency); Broken line—slides 3 and 4 (low chiasma frequency).

Chiasma pairs across the attachment

Slides 3 and 4 were from material grown at a higher temperature (18–22°C) than the other material. In them the region of the attachment is clearly split in some of the chromosomes (see fig. 3). Ordinarily it is impossible to distinguish the type of a chiasma pair which includes the attachment, owing to the impossibility of tracing the strands through it. In sixteen of the bivalents, however, both attachment regions were split

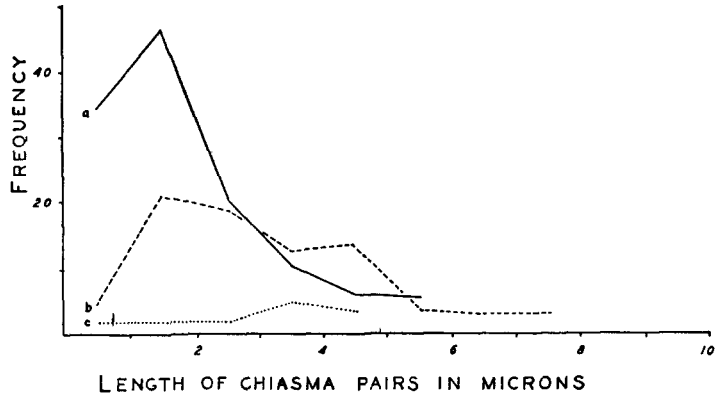

FIGURE 5. Numbers of types a, b, and c in the different size classes. (Slides 1 and 2; from Table 6).

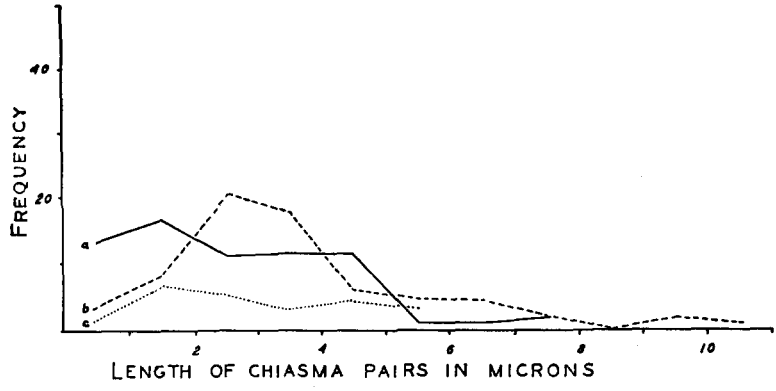

FIGURE 6.—Numbers of types a, b, and c in the different size classes (Slides 3 and 4; from Table 6). Note: One type b configuration in size class 12–13 was observed but has been omitted from the graph.

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	DIFF. OF L. / σ_d	P
a–b	1.156	0.169	6.8	.000,000,001
a–c	1.107	0.265	4.2	.000,01
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	.03
b–d	0.989	0.601	1.6	.11
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

so clearly that the strands could be traced through them with a fair degree of accuracy. 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 5). This is not a sufficiently large number from which to determine whether the attachment has affected the proportions of the types.

TABLE 5

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

In case there should be a noticeable difference between those in which one, both, or neither of the chiasmata is close to the attachment, the numbers are given separately in table 5 for each of these three classes. The data, though far too limited for significance, suggest that in chiasma pairs straddling the attachment the proportion of type a may possibly be greatest where both chiasmata are adjacent to the attachment, making the interstitial distance short. This would be in agreement with the data obtained from the arms of the chromosomes. However, 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 cases in which the strands could be traced through the attachment region.

Effect of crowding of chiasmata upon the types formed

Slides 1–4 differ considerably in chiasma frequency, 1 and 2 having an average of 22.2 chiasmata per cell, and 3 and 4 an average of 15.8. It is of interest to note the effect which differences in chiasma frequency have

upon the proportions of the different types of chiasma pairs. It may be seen in figure 4 that the greater chiasma frequency of slides 1 and 2 has resulted in a crowding of the chiasmata and an increased number of chiasma pairs with an interstitial length of 0–1 and 1–2 microns and a slight but consistent decrease, relative to slides 3 and 4, in the number of chiasma pairs with interstitial lengths greater than this. The data from which figure 4 was derived are presented in table 6.

TABLE 6

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

From the length-complexity-frequency correlation found 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; 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 6).

From table 6 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 shortest length class, 0–1μ, type a is greatly in excess in both cases. In the 1–2μ length class type a is about twice as frequent as type b, in both pairs of slides. In some of the length classes (especially class 4–5μ) differences in the proportion of types a and b do occur between the two pairs of slides, but since the numbers are small these differences are probably not significant.

DISCUSSION

Definite results of the cytological study and their relation to genetic data on crossing-over

All chiasma pairs in which the chromatids could clearly be traced can be fitted into eight basic types (a–h). As shown in figure 1 eight configurations which would look different under the microscope could contribute to any one of the basic types except a and c, which owing to their symmetry comprise only four. All four have been found in type a and all eight in type b. In type c neither the indistinguishable aspects 7 and 8 nor their mirror images were found; these are those with the greatest number of twists. In addition mirror images of type b were found corresponding to aspects 1–7 and mirror images would be expected for types e, f, g, and h, if they were sufficiently numerous. At first glance there are apparent similarities between some of the one-plane representations of different basic types. For example, a 5–8 may look like c 1–4 and a 1–4 like c 5 and 6, but all of type a are "free" configurations, while all of type c have interlocked chromatids. Similarly types c and d are in general superficially alike but type d are "chromosome locks" having one chromosome lying between the two chromatids of the other.

The observations show definitely that a certain amount of chromatid twisting must exist at the time of chiasma formation as in the more complex types of chiasma pairs it could not have arisen later. However, it is not possible to say how many twists a particular type has since it may present so many aspects under the microscope or be represented in so many different ways. When all possible representations are considered, however, we can say that certain types tend to have more twists than others, and that there is clearly a general correlation between interstitial length and complexity, as shown in figure 1.

The simplest types are definitely the most frequent. But judged either by number of chromatid overlaps or changes in level, types c and d are of equal complexity, yet type c occurs twice as frequently as type d. There are various assumptions by which this may be explained. Neglecting these for the present, there is in any case general covariance of frequency, interstitial length and complexity: see figure 2, and tables 1, 2, 4 and 6.

We may for statistical treatment group all the configurations into two classes as compensating and non-compensating chiasma pairs since this is the classification most definitely of genetic significance. In the preliminary Trillium data the average distance between chiasmata in 33 pairs of type a was 2.3μ and in 15 of type b it was 3.3μ. Using a fourfold table, the association between length and type was found to be highly significant; $\chi^2 = 6.7$; $P = .01$. In the Trillium main data the average dis-

tance was 2.5μ in type a and 3.7μ in type b. It was 2.8μ in the total of 244 compensating pairs (types a, c, d, e, and h) and 3.7μ in 147 non-compensating pairs (types b, f, and g). There is no apparent terminalization and its only effect if present would be a partial masking of the original differences in length. Analysis of HEARNE and HUSKINS' Melanoplus figures shows that the average distance between chiasmata in 39 pairs of the compensating types, a, c, and d, is 3.8μ and in 26 non-compensating pairs, type b, it is 4.8μ. Differences in interstitial length between types a and b are shown with their statistical significance in table 3. In table 4 the differences between all the types are presented.

WEINSTEIN (1936) has concluded from analysis of extensive Drosophila data that "the chromatids that cross over at one level do not determine which ones cross over at other levels." This implies that two-, three-, and four-strand double crossovers will occur in the ratio of 1:2:1. Three-strand doubles correspond to the continuous or non-compensating type observed cytologically. The two- and four-strand doubles cannot (without making unverified assumptions) be distinguished cytologically and have collectively been termed compensating chiasma pairs. In the absence of some form of "chromatid interference" in chiasma formation compensating and non-compensating pairs would be expected in equal numbers. In Trillium slides 1 and 2 there are 139 compensating pairs, and 79 non-compensating pairs. In slides 3 and 4 there are 105:68. In HEARNE and HUSKINS' analysis of Melanoplus there were 71 compensating and 35 non-compensating pairs. Thus in both Trillium and Melanoplus there is a great deficiency of non-compensating pairs. Evidently the association of chromatids at one end of a chiasma pair is not independent of that at the other in these materials; there is cytological evidence of chromatid interference. Further, the association between interstitial length and type of chiasma pairs shows that chromatid interference varies with the distance between chiasmata. If, despite general opinion to the contrary, cancellation of chiasmata can occur before anaphase, the higher chiasma frequency slides must give a truer picture of the extent of chromatid interference.

LINDEGREN and LINDEGREN (1937) have obtained genetic evidence from Neurospora indicating that recurrence of crossing-over is not a random process. The ratio of two-, three-, and four-strand doubles obtained was 27:14:8. Thus they have evidence of "negative chromatid interference." These data involved the attachment as a point of reference. For analysis they divided the chromosome into four regions with the attachment between regions 1 and 3. Between adjacent regions 1–2 and 3–4 seven two-strand, four three-strand and three four-strand doubles occurred. Between regions 1–3 and 2–4 there were 0, 4, and 1. Between regions 1–4 there were 10, 6, and 3. Between regions 2–3 there were 10, 0 and 1. There is, thus, a

greater proportion of two-strand doubles in double exchanges involving adjacent regions than in those separated by one region. Though the numbers are very small it looks as if there may be some relationship between interstitial length and type of double crossover analogous to the length-complexity relationship of chiasma pairs in Trillium. The strand relationships across the attachment are, however, possibly reversed in Neurospora and Trillium (see table 5); in neither case are the numbers large enough to determine significance. Neurospora and Trillium agree in showing a great deficiency of three-strand doubles relative to two-strand and four-strand combined. In Neurospora the latter two classes can be distinguished and there appears to be a significant excess of two-strand doubles (27:8). Though they cannot definitely be distinguished in Trillium, it will be shown in the next section of this discussion that if certain plausible but unverifiable assumptions are correct there is a similar excess in Trillium.

LINDEGREN and LINDEGREN (1939) have further data. In the chromosome studied, all the genes were, however, on one side of the attachment, which was itself used as a marker. Under such conditions it would seem impossible to distinguish between two- and four-strand double crossovers as they attempted to do. 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 compensating to 17 non-compensating chiasma pairs. In the previous Neurospora data the ratio was 35:14. Both these ratios deviate from the 1:1 ratio in the same direction as the cytological data from Trillium and Melanoplus, but the first is, of course, not significant.

A re-analysis we have made of BEADLE and EMERSON'S (1935) attached-X Drosophila data indicates that their observations do not necessarily prove crossing-over to be a random process at successive "levels" as they assume. In fact, a length-type analysis seems to suggest that there may be a direct correlation between the genetic length of chromosome separating two crossovers and the proportion of two-strand and three-strand doubles (see fig. 7). Short lengths seem to be associated with an excess of two-strand doubles. The very small number of individuals in the critical classes, that is, the short double exchanges, must, however, be noted.

When this paper was presented at the Seventh International Genetics Congress the question was raised whether the 391 chiasma pairs which could be analysed in detail were a random sample of the total of 508 observed in the 48 complete nuclei. This point already had been considered and the conclusion reached that they probably were since the ease with which configurations could be traced seemed to depend almost entirely upon clarity of fixation rather than upon lack of complexity. In any case,

it was emphasized, there is in the main data an excess of 93 compensating pairs and to raise the non-compensating pairs to equality, it would be necessary to make the most improbable assumption that nearly all the 117 pairs which could not be analysed were of the latter type. That this assumption is quite unwarranted for the shorter size classes is readily proved by showing that within the $0-1\mu$ class the proportion of compensating to non-compensating pairs is 50:7 and in the $1-2\mu$ class it is 75:31 while the number of pairs which could not be analysed was only 4 and 25 in these two size-classes respectively. Further, 23 of the latter 25 were on two slides (14 on slide 1 and 9 on slide 2) which increases the probability that in these short lengths clarity of fixation is the chief limiting factor.

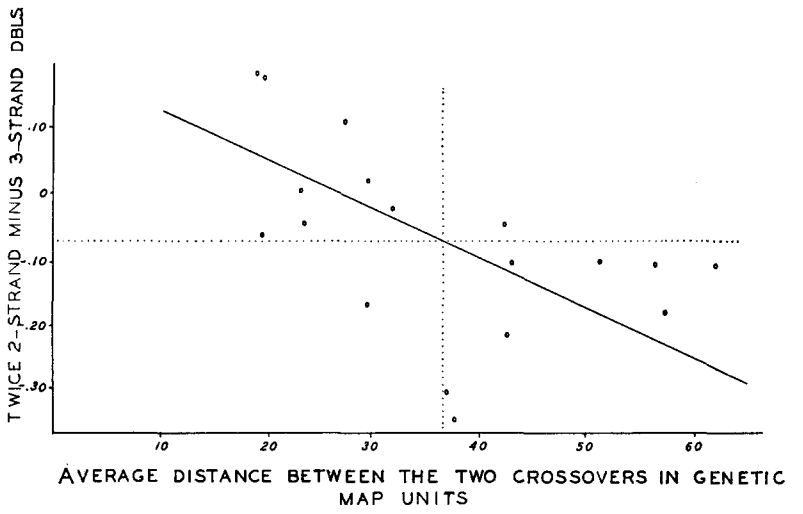

FIGURE 7.—Excess of two-strand doubles in Drosophila over the number expected on a random basis (from the attached-X data of BEADLE and EMERSON, 1935, table 7).

It remained probable, however, that, other things being equal, pairs of chiasmata with short interstitial distances would be easier to classify than the longer ones. An analysis was therefore made of the frequency distribution of the compensating, non-compensating and unclassified pairs (table 7 and fig. 8). From this it is clear (see especially the percentage unclassified in size-classes up to 7μ) that length is a factor in ease of analysis and that the distribution of the unclassified pairs is significantly different from that of the classified pairs as a whole. The distribution is more like that of the non-compensating than that of the compensating pairs. This is, of course, expected if length is a factor in ease of analysis, since the mean length of the non-compensating pairs (3.7μ) is greater than that of the compensating (2.8μ). In the longest and most complex types, how-

ever, two (e and h) are compensating and two (f and g) are non-compensating; their mean lengths should therefore be similar. In brief, there is a suggestion that the unclassified pairs may include somewhat more non-compensating than compensating types. This cannot be proved or disproved and there is certainly nothing to suggest that they are largely non-compensating types.

TABLE 7

Frequency distribution of unclassified and classified chiasma pairs in relation to interstitial length.

		SIZE CLASSES IN MICRONS													
	SLIDE	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
Unclassified	1	1	9	3	7	6	5	2	0	2	1	0	0	0	36
	2	3	14	12	12	14	3	2	0	1	0	1	0	0	62
	3	0	1	2	5	3	1	0	0	0	0	0	0	0	12
	4	0	1	1	2	1	1	0	1	0	0	0	0	0	7
	1 and 2	4	23	15	19	20	8	4	0	3	1	1	0	0	98
	3 and 4	0	2	3	7	4	2	0	1	0	0	0	0	0	19
	Total	4	25	18	26	24	10	4	1	3	1	1	0	0	117
Non-comps.	1 and 2	4	22	19	13	13	3	2	2	0	1	0	0	0	79
Non-comps.	3 and 4	3	9	20	17	6	4	3	2	0	2	1	0	1	68
Non-comps.	1–4	7	31	39	30	19	7	5	4	0	3	1	0	1	147
Comps.	1 and 2	35	51	23	15	9	6	0	0	0	0	0	0	0	139
Comps.	3 and 4	15	24	18	15	18	4	3	6	1	1	0	0	0	105
Comps.	1–4	50	75	41	30	27	10	3	6	1	1	0	0	0	244
	Total	57	106	80	60	46	17	8	10	1	4	1	0	1	391
Percentage Unclassified		6.6	19.1	18.4	30.2	34.3	37.0	33.3	9.1	75.0	20.0	50.0	—	0.0	23.0

Figure 8 shows very strikingly that whatever the proportion of compensating and non-compensating types in the unclassified pairs there cannot be equality of these types throughout the range of the size classes. The main conclusions of this paper therefore remain quite unchanged whatever type the unclassified pairs are assumed to be. These conclusions are that frequency, complexity, and interstitial length of the chiasma pairs show covariance and that there is therefore some type of "chromatid interference."

Ignoring the distribution of the cytological types with regard to length of chromosome and making two assumptions: (1) that almost all unanalysed pairs are non-compensating, and (2) that compensating pairs consist of two-strand and four-strand doubles in equal numbers, it would be possible to harmonize these data with a random (1:2:1) proportion of two-, three-, and four-strand double exchanges in the total data with the chromosomes considered as units, but this ratio would not occur in

all segments of the chromosome—it is clearly established that it does not occur within short regions. It is interesting that our length-type analysis of published Drosophila data suggests that this situation may possibly occur in it. Evidently further studies of Drosophila with this factor in mind are needed.

The cytological data considered on the basis of various assumptions

Throughout the foregoing analysis we have assumed that the chromosomes and the paired chromatids can move in any direction in the process

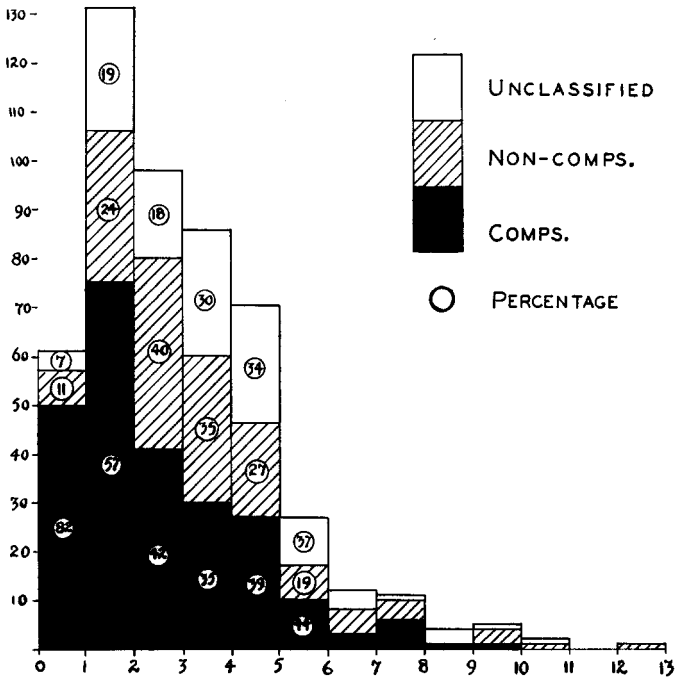

FIGURE 8.—The percentage distribution of unclassified, classified, compensating and non-compensating chiasma pairs, in different size classes (from table 7).

of opening-out after chiasma formation and that the angle from which they are viewed may affect the aspect presented and therefore the representation of a particular type. This accounts for the eight possible representations (apart from mirror images of b, c, f, g and h) of each type. This assumption has been made because it is impossible to prove that they do not move at random and any conclusions based on any other assumption must at present therefore rest on insecure foundations. If for the moment we postulate, however, that movement is not random in opening-out and that in a fairly large series of observations the angle of vision factor will be randomly distributed, we may analyse the configurations further. We

may classify the original drawings of the chiasma pairs according to the scheme of figure 1 instead of being limited to classification into the eight distinct types. Analysing the configurations on this basis we obtain the results shown in table 8. In this table the most frequent types, a–d, are each subdivided into two groups (rows 1–4 and 5–8 of fig. 1) and types b and c are further subdivided into their mirror images. The direction of crossing of the chromatids (the "chiasma direction"—DARLINGTON 1937) is given at chiasmata successively distant from the attachment with the direction considered, for the purpose of analysis, as a twist proceeding away from it. In rows 1–4 the chiasmata are opposite, LR or RL, in all eight types and in rows 5–8 they are all "in the same direction," LL or

TABLE 8

"Chiasma direction" and assumed types of double crossovers.

TYPE (*cf.* fig. 1)	CHIASMA DIRECTION: OPPOSITE LR	OPPOSITE RL	SAME LL	SAME RR	ASSUMED TYPE OF CROSSOVERS: 2-STRAND	3-STRAND	4-STRAND
a1–4	64	72			136	0	0
a5–8			4	1	0	0	5
b1–4 (1 and 2)	60 (34)	36 (15*)			0	96	0
b1–4 (3 and 4)	60 (26*)	36 (21)					
b5–8 (5 and 6)			12 (4)	13 (5*)	0	25	0
b5–8 (7 and 8)			12 (8*)	13 (8)			
c1–4 (1 and 2)	7 (4)	6 (3*)			0	0	13
c1–4 (3 and 4)	7 (3*)	6 (3)					
c5–8 (5 and 6)			10 (10)	7 (7*)	17	0	0
c5–8 (7 and 8)			10 (0*)	7 (0)			
d1–4	7	4			0	0	11
d5–8			3	0	3	0	0
	138	118	29	21	156	121	29

* Mirror images of the interpretations shown in figure 1; not distinguishable in types a or d

RR. We see from table 8 that an overwhelming proportion of chiasmata are in opposite directions in type a configurations (136 LR or RL:5 LL or RR). In type b there is still a great, though smaller, excess of "opposite" pairs (96:25). In types c and d the proportions do not deviate significantly from equality. It should be noted that mirror images (viewed, of course, from above or below to retain the proximal-distal orientation) have reversed chiasma directions (left becomes right and *vice versa*) and that movement of the chromosome ends in the same direction on either side of a chiasma pair would give opposite chiasmata in type a but chiasmata in the same direction in types c and d, *cf.* figure 1. The significance of the great total excess of opposite twisting at successive chiasmata cannot at present be determined. It is possible that some small part of the excess

may not be real, as when one chromatid lies directly over another there may be a tendency on the part of the observer to draw the configuration according to the simpler of the alternative interpretations then possible. This was not at first realized; in the final analysis of the drawings many doubtful cases have been omitted to reduce this possible source of error.

If we consider that opening out at diplotene and later does not involve a rotation of the chromosome ends then one simple assumption to explain the great excess of opposite pairs of chiasmata in types a and b would be that crossing-over in the short regions which these involve is usually conditioned by chromosome overlaps, not by torsional strain. Another simple assumption to explain the excess of opposite pairs without rotation is that they result directly from a tendency of alternate internodes to open out at right angles to each other. A recent recheck of the observations (S. G. SMITH unpublished) does not, however, entirely support this widely accepted assumption.

If we consider, on the other hand, that "relational coiling" conditions crossing-over, we have two possibilities depending upon whether crossing-over relieves torsion completely or only partially. If completely, there would be no need for rotation in opening out and the excess of opposite pairs would be unexpected. If, however, torsion is still present after crossing-over and there is rotation in opening out, then the excess of opposite pairs in types a and b may be due merely to untwisting being more complete in these shorter types.

Further studies, especially of structurally aberrant bivalents and of relational coiling may enable decisions to be made regarding these and other possibilities. For the present we can say only that on the initial postulates of this section of the discussion the distance separating a pair of chiasmata is related to the tendency for opposite or similar twisting at successive chiasmata. The similarity of this to the relation between frequency and length in the analysis of the eight types of chiasma pairs deserves further consideration.

Following further this analysis in terms of the figure 1 representations of each pair of chiasmata and assuming in addition that crossing strands at a chiasma are consistently *either* the genetic crossovers or the non-crossovers we find that in 306 pairs of types a–d, with each type subdivided into the two groups with opposite and similar chiasma directions, there are 156 two-strand, 121 three-strand and 29 four-strand double exchanges (table 8). This deviates from randomness in the same direction as the LINDEGRENS' genetic ratio of 29:14:8 in Neurospora. For a further possible analysis of the configurations in terms of crossing-over we may revert, as in the first part of this discussion, to consideration of only the eight distinct types without any attempted subdivision of each of these.

We can then include representations that fall into both of the sub-groups 1–4 and 5–8. On any of the interpretations of the chiasma theory of crossing-over type b configurations must be three-strand doubles. There are 144 of these in the total of 384 configurations of types a–d, whereas in the absence of chromatid interference there should be 192. We cannot distinguish two-strand from four-strand doubles. But let us assume for the moment that type a pairs are two-strand doubles and types c and d both four-strand doubles. This assumption though without any verifiable foundation is a very natural one, since out of 141 type a configurations 136 fall into rows 1–4, that is, they appear under the microscope as if only two strands were involved in the two chiasmata. Analysed on this basis the 384 configurations of types a–d comprise two, three and four-strand doubles in the ratio of 190:144:50. Again it deviates very significantly from the expected 1:2:1 and this time it is strikingly similar to the Neurospora genetic ratio. Such similarity cannot, however, be considered too seriously, even apart from the unverified postulates involved, since on the one hand the number of Neurospora data is small (in one case too small for significance), and on the other the proportions of the different types are different in the two lots of Trillium ($P = <.01$) though the difference between them in their proportions of compensating and non-compensating pairs is not significant ($P = >.5$).

On the assumptions of the preceding paragraph the statement which has been made on the basis of the differences in length of type a and b configurations, that chromatid interference differs in different length classes, can be extended to a statement that a decrease in chiasma frequency decreases the amount of negative chromatid interference. Comparing slides 1 and 2 with 3 and 4 there is a drop of type a from 122 to 68, a change only from 77 to 67 in type b, while types c and d together increase from 16 to 34. In other words, on these assumptions slides 1 and 2 have two-, three- and four-strand doubles in a ratio of 122:77:16 while in slides 3 and 4 it is 68:67:34; the difference being highly significant.

CONCLUSIONS

We conclude that chromatid interference occurs but that it is not simply a matter of a "force" which influences the chance which a particular strand has of being involved in both of two successive chiasmata or crossovers. Rather one may think of the proportion of two-, three-, and four-strand doubles as being determined by the complexity of the various configurations from which each can arise. The complexity is related to chiasma frequency, type frequency and interstitial length. The simplest configurations are those most frequently produced, and the shorter the distance between two chiasmata the greater the relative frequency of the

simpler types. There are thus certain variables regularly associated with the cytological types of chiasma pairs and therefore with the genetic types to which these contribute.

Both chiasma and chromatid interference appear to be integral and correlated parts of a complex process as yet inadequately analysed. The data provide positive evidence on points not previously considered in either cytological or genetical analyses, and show the inadequacy or error of existing theories on the mechanism of crossing-over. They indicate that further studies are needed on Neurospora and other such genetic materials and on attached-X Drosophila, with more numerous and better distributed markers. If crossing-over is a more complex process than has previously been recognized, it follows that restricted hypotheses that aid in the elucidation of specific parts of the problem are necessary, but that any attempt to formulate a general hypothesis to explain the *mechanism* must be highly speculative until more detailed knowledge of its *results* is available. We have not therefore presented here in detail all the various analyses that have been made of the data in terms of existing theories of crossing-over or of adaptations and modifications of them that have been devised in the course of the work.

ACKNOWLEDGMENT

As the junior author is in England it has not been possible for him to collaborate fully in the final preparation of this paper. In his absence, DR. S. G. SMITH has kindly made many statistical analyses and given much assistance for which we desire to express our thanks.

SUMMARY

In preparations of pollen mother cells of *Trillium erectum* in which it is possible to trace the spatial relationships of all four chromatids of the first meiotic metaphase bivalents, chiasmata have been analysed in 48 complete cells (240 bivalent chromosomes). Eight distinct types (a–h) of pairs of chiasmata were found. Movements of chromosome ends and differences in the angle of vision may produce as many as eight (1–8) one-plane representations of each of these types—apart from mirror images.

Where mirror mages are distinguishable (in types b, c, e, f, g, and h) there are 106 of one and 78 of the other.

The simplest types of chiasma pairs, whether complexity is judged by number of chromatid overlaps or "changes of level," are produced most frequently and there is a general inverse relationship between complexity and frequency.

Mean distance between chiasmata taken in pairs varies with complexity.

The length-complexity relationship is consistent in preparations varying in chiasma frequency. High chiasma frequency and consequent short mean interstitial length is correlated with higher proportion of the simpler types of chiasma pairs.

Compensating pairs of chiasmata are greatly in excess in both high and low chiasma frequency preparations and a 2:1 ratio had previously been found in Melanoplus.

Chiasma pairs across the attachment appear to show an excess of the non-compensating type but the number is too small to determine whether the proportion deviates significantly from randomness or from that observed in the arms.

Genetic analyses of Drosophila have been interpreted as showing that "the chromatids that cross over at one level do not determine which ones cross over at other levels." Neurospora genetic data show chromatid interference. Reanalysis of certain published Drosophila data indicates that in it there may be a length-complexity relationship which has not previously been considered. Non-compensating pairs of chiasmata represent three-strand double crossovers on any interpretation of the chiasmatype theory. Assuming only that chiasmata represent crossovers, the deficiency of non-compensating pairs indicates that in Trillium and Melanoplus some form of chromatid interference occurs in crossing-over.

Out of a total of 508 pairs of chiasmata 117 could not be classified. It is shown that the unclassified pairs may possibly contain somewhat more non-compensating than compensating types, but there is nothing to suggest that there is any great excess of the former.

Whatever the constitution of the unclassified group, the main conclusions are unaltered. They are that compensating pairs of chiasmata are more numerous than non-compensating, that interstitial length and complexity of strand relationships in a pair of chiasmata vary together and are inversely related to the frequency with which a type of chiasma pair occurs. It follows that there must be some form of "chromatid interference" in chiasma formation. This cytologically determined interference cannot be described in such simple terms as negative or positive chromatid interference, which have been used to describe differences in the proportion of genetic types. That these are in any case arbitrary terms from the cytological point of view is clear from the fact that the genetic types are of mixed origin from different kinds of chiasma pairs. There is no cytological evidence for chromatid interference between successive chiasmata which are widely separated; chromatid interference like chiasma interference is therefore a function of distance and of chiasma frequency.

LITERATURE CITED

BEADLE, G. W. and EMERSON S., 1935 Further studies of crossing over in attached-X chromosomes of *Drosophila melanogaster*. Genetics **20**: 192–206.

BELLING, JOHN, 1929 Nodes and internodes of trivalents of Hyacinthus. Univ. Calif. Pub. Bot. **14**(18): 379–388.

BONNIER, G. and NORDENSKIÖLD, M., 1937 Studies in *Drosophila melanogaster* with attached X:s. I. Crossing-over values. Frequencies of reciprocal and non-reciprocal exchanges. Chromatid interference. Hereditas **23**: 258–278.

DARLINGTON, C. D., 1930 A Cytological Demonstration of "Genetic" Crossing-over. Proc. Roy. Soc. B., **107**: 50–59.

1937 Recent advances in Cytology. 2d Ed. Philadelphia: Blakiston.

HALDANE, J. B. S., 1931 The cytological basis of genetical interference. Cytologia **3**: 54–65.

HEARNE, E. M. and HUSKINS, C. L., 1935 Chromosome pairing in *Melanoplus femur-rubrum*. Cytologia **6**: 123–147.

HUSKINS, C. L. *et al.*, 1938 Chromonema and chiasma studies in asynaptic, desynaptic and normal *Trillium erectum*. Genetics **23**: 152.

HUSKINS, C. L. and SMITH, S. G., 1935 Meiotic chromosome structure in *Trillium erectum* L. Ann. Bot. **49**(193): 119–150.

HUSKINS, C. L. and WILSON, G. B., 1938 Probable causes of the changes in direction of the major spiral in *Trillium erectum* L. Ann. Bot. New Series **2**: 281–292.

LINDEGREN, C. C. and LINDEGREN, G., 1937 Non-random crossing-over in Neurospora. J. Hered. **27**: 105–113.

1939 Non-random crossing-over in the second chromosome of *Neurosopora crassa*. Genetics **24**: 1–7.

MATHER, K., 1938. Crossing-over. Biol. Rev. **13**: 252–292.

MATSUURA, H., 1940 Chromosome studies on *Trillium kamtschaticum* Pall. XII. The mechanism of crossing-over. Cytologia **10**(3): 390–405.

SAX, K., 1936 Chromosome coiling in relation to meiosis and crossing-over. Genetics **21**: 324–338.

WEINSTEIN, A., 1936 The theory of multiple-strand crossing-over. Genetics **21**: 155–199.

WILSON, G. B., and HUSKINS, C. L., 1939 Chromosome and chromonema length during meiotic coiling in *Trillium erectum* L. Ann. Bot. New Series **3**: 257–270.