CHIASMA INTERFERENCE IN TRILLIUM ERECTUM

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INTRODUCTION

IT has been shown by BELLING (1929), DARLINGTON (1930), and others that the chiasmata in certain cytological configurations must have been accompanied by genetic crossing-over. However, it is not yet fully established that all chiasmata represent crossovers. To demonstrate the complete concomitance of chiasma formation and crossing-over it is necessary to show them to be similar in as many different ways and under as many different conditions as possible.

It has been shown that chiasma formation and crossing-over may be similarly affected by environmental factors both internal and external. For reviews see DARLINGTON (1937) and MATHER (1938). Further, HALDANE (1931) has shown that interference similar in one respect to that between crossovers occurs also between chiasmata. He found that variability in the number of chiasmata per bivalent is less than would be expected were chiasmata independent of one another in their formation. However, a detailed analysis of cytological interference can be made only by dividing the chromosomes into regions similar to those separated by genetic markers and recording accurately the positions of individual chiasmata in these regions. This was not possible with the observational data used by Haldane.

Such a study has been carried out with *Trillium erectum* on preparations which show clearly the space relationships of the four chromatids in each bivalent (HUSKINS and NEWCOMBE 1941). Further, this material has (1) long chromosomes, (2) a relatively high chiasma frequency, (3) only five bivalents, each of which may be identified by its length and the position of its attachment region, and (4) no indication of terminalization or other movement of chiasmata during prophase (although the possibility that minor changes in position do occur cannot be entirely ignored).

MATERIALS AND METHODS

The preparations were those used by HUSKINS and NEWCOMBE (1941). In this material coiling is reduced, and the chromatids are clearly differentiated. This latter is particularly important, since in ordinary bulk-stained preparations it is often impossible to determine whether a region of contact between homologous chromosomes represents one or two chiasmata. In studying interference it is, of course, of the utmost importance

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that when two chiasmata occur close together they shall not be mistaken for a single chiasma.

In order to obtain values of chiasma coincidence in *Trillium*, each chromosome of the complement was divided into a definite number of sections equal to approximately one micron. The sub-terminally-attached A chromosome, for instance, varies in length in different cells and preparations from 10 to 17 μ . Since the average length was 12.2 μ in our material, each individual A chromosome was divided into twelve equal regions regardless of its length. In a chromosome of average length each region would be 1.02 μ long. Each arm of the four remaining chromosomes, all of which have median or sub-median attachments, was treated in a similar manner. Thus the attachment was made to separate two regions rather than to occur within one of them.

It has thus been possible to divide the *Trillium* chromosomes into a large number of regions: from 12 in the A chromosome to 21 in the E chromosome. This is a much greater number than can be marked genetically in *Drosophila*. In a general sense then, the positions of all the chiasmata occurring in a particular bivalent have been located more accurately than can all the crossovers occurring in an individual chromosome of any genetic experiment. In other respects there are certain limitations to a cytological study of crossing-over (*cf.* HUSKINS and NEWCOMBE 1941).

The formula used for calculating coincidence was: coincidence = xn/ab in which x is the number of double chiasmata, n is the total number of pairs of regions observed, and a and b are the numbers of chiasmata in the first and second regions, respectively, including those occurring simultaneously in both. The suggestions of STEVENS (1936) regarding the use of this formula for non-adjacent regions were adopted.

The comparisons between the coincidence values obtained from different chromosomes, or different parts of the same chromosome, are not subject to errors resulting from differences in the distances separating crossovers. This is a difficulty in genetic experiments, since coincidence varies with distance, and it is not possible to space marker genes to provide a large number of regions of equal genetic or cytological length.

Twelve cells were analyzed from each of four preparations; in these 48 cells only two bivalents (two C bivalents in slides 1 and 2) could not be interpreted. These are omitted from all calculations, and this omission is responsible for the slight differences which may be noted in the tables between the values of n for the two sets of slides.

Slides 1 and 2 had a higher chiasma frequency (21.3 and 23.0 chiasmata per cell, respectively) and slides 3 and 4 a lower chiasma frequency (14.3 and 17.4 chiasmata per cell, respectively). The data from the two pairs of slides were analyzed separately throughout the study.

OBSERVATIONS

The frequencies of chiasma formation for the various regions are given in table 1. Coincidence values were first calculated for adjacent regions of one micron. There is a high frequency of chiasma formation close to the attachment, and a considerable number of data may be obtained from the two regions adjacent to it in any one of the four bivalents having median

TABLE 1
Distribution of chiasmata in the five bivalents of Trillium.

CHROMO- SOME SLIDES	NUMBER OF BIVALENTS EXAMINED	REGIONS* OF THE LEFT ARM										ATTACH- MENT	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)	24				3	6	3	4	5	4	15		14	3	3	3	8	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	

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

or sub-median attachments. Within the arms, where the chiasma frequency is much lower, it is necessary to combine all the data from a comparison of each region with its neighbor in order to get an estimate of coincidence for that arm. Differences in chiasma frequency in different parts of the arm would in themselves make this estimate somewhat higher than the average of the coincidence values from each pair of regions were sufficient data available to calculate them individually. The discrepancy, however, affects the usefulness of these values in only one of the comparisons made. This will be considered in the discussion.

The data and coincidence values obtained from adjacent regions across the attachments are given in table 2 and the statistical analysis in table 3. In all bivalents coincidence across the attachment is greater than unity, and it is significantly greater in the data from slides 3 and 4 and in the combined data from all slides.

Coincidence across the attachment is greater in slides 3 and 4 (which have a lower chiasma frequency) than in slides 1 and 2. This difference is just significant, however: $P = .05 - .02$.

There is no indication of any difference between the attachments of bivalents B, C, D, and E with regard to the coincidence values across each.

Coincidence values between adjacent one micron regions not separated by the attachment are given in table 4, and the statistical analysis in table 5. Contrary to the behavior of coincidence across the attachment, this is greater in slides 1 and 2 (which have a higher chiasma frequency)

TABLE 2
*Chiasma data and coincidence values for adjacent one micron regions separated by the attachment.**

CHROMOSOME	a	b	x	n	c	V(c)	χ^2
Slides 1 & 2							
B	16	18	14	24	1.167	0.012	2.286
C	15	16	12	22	1.100	0.011	0.949
D	19	24	19	24	—	—	—
E	19	23	19	24	1.043	0.002	1.000
Weighted mean					1.065	0.001	3.025
Slides 3 & 4							
B	7	11	6	24	1.870	0.165	4.598
C	14	12	9	24	1.286	0.036	2.263
D	15	14	11	24	1.257	0.025	2.610
E	19	14	13	24	1.173	0.012	2.431
Weighted mean					1.242	0.006	9.076

* a & b are the numbers of chiasmata in the two regions compared respectively, and include those occurring simultaneously in both regions; x is the number of double chiasmata in the two regions; n is the total number of observations; c is the coincidence; V(c) is the variance of the coincidence (from formula given by Stevens 1936); χ^2 is calculated for the deviation from unity. Deviation of c from its expectation squared, divided by the variance is distributed approximately as a χ^2 for one degree of freedom. If in the calculation of the variance c had been placed equal to its expected value of one, this quantity would be a perfect χ^2 . The discrepancy involved in the above calculation, however, is not likely to cause serious misjudgment.

TABLE 3
Analysis of coincidence across attachment.

	D.F.	χ^2	P
Deviation from unity	1	8.137	< .01
Heterogeneity between Slides 1 & 2 and 3 & 4	1	3.964	.05 — .02
Heterogeneity within Slides 1 & 2 and 3 & 4	5	4.056	.70 — .50
Total	7	16.157	

than in slides 3 and 4. The difference in this case is not statistically significant, however: $P = .10 - .05$.

A test was made to determine the change in coincidence when the regions compared were separated by from zero to six other regions. Data from all

the chromosome arms were combined, and slides 1 and 2 and 3 and 4 were treated separately. It may be seen from table 6 that, in general, coincidence is greater when the regions are more widely separated. Statis-

TABLE 4
*Chiasma data and coincidence values for adjacent one micron regions
not separated by the attachment.*

CHROMOSOME	a	b	x	n	c	V(c)
High chiasma frequency Slides 1 & 2						
A	56	51	4	264	0.370	0.029
B (l)	7	19	0	72	—	—
II (r)	77	66	17	216	0.723	0.017
C (l)	12	26	3	88	0.846	0.157
(r)	44	36	8	132	0.667	0.034
D (l)	34	52	8	144	0.645	0.033
(r)	77	59	17	168	0.628	0.012
E (l)	35	49	6	216	0.756	0.069
(r)	75	59	8	240	0.433	0.018
Low chiasma frequency Slides 3 & 4						
A	42	45	0	264	—	—
B (l)	3	9	0	72	—	—
(r)	49	41	4	216	0.430	0.038
C (l)	12	20	0	96	—	—
(r)	29	19	0	144	—	—
D (l)	25	37	3	144	0.467	0.058
(r)	39	29	2	168	0.297	0.039
E (l)	45	57	5	216	0.421	0.028
(r)	54	51	6	240	0.523	0.035

TABLE 5
Analysis of coincidence between adjacent regions within the arms.

	DF	χ^2	P
Heterogeneity between slides 1 & 2 and 3 & 4	1	2.754	.10-.05
Heterogeneity within slides 1 & 2 and 3 & 4	11	6.076	.90-.80
Total	12	8.830	

tically significant indications of interference are found when the regions are separated by as much as two microns. When farther apart, coincidence does not differ significantly from unity.

DISCUSSION

Evidence that coincidence across the attachment may exceed unity is found in the genetic data from chromosome III of *Drosophila melanogaster* and chromosome I of *Neurospora crassa*. KIKKAWA (1935) has calculated coincidence from the relevant *Drosophila* data of BRIDGES and MORGAN (1923), MULLER (1925), BRIDGES (1929), and REDFIELD (1930). The one group of these data which gives a coincidence value significantly greater than one is included in table 7 together with its χ^2 . Also, coincidence values have been calculated by the author from the *Neurospora* data of LINDEGREN and LINDEGREN (1937) and are given in table 7 together with the χ^2 's for their deviations from one.

TABLE 6
Coincidence when regions within arms are separated by different distances.
(Stevens' (1936) formula used.)

DISTANCE BETWEEN REGIONS	a	b	x	n	c	V(c)	χ^2
Slides 1 and 2							
0 μ	417	417	71	1540	0.629	.0038	36.657
1 μ	372	371	95	1328	0.914	.0048	0.838
2 μ	333	337	84	1116	0.835	.0046	5.946
3 μ	284	279	81	904	0.924	.0053	1.091
4 μ	236	205	77	716	1.140	.0073	2.651
5 μ	176	153	50	550	1.021	.0101	0.045
6 μ	124	95	35	384	1.141	.0173	1.146
Slides 3 and 4							
0 μ	298	308	20	1560	0.340	.0050	87.296
1 μ	271	268	44	1344	0.814	.0105	3.300
2 μ	235	244	52	1128	1.023	.0124	0.043
3 μ	204	207	48	912	1.037	.0132	0.102
4 μ	167	149	33	720	0.954	.0172	0.118
5 μ	139	124	26	552	0.833	.0169	1.725
6 μ	99	82	19	384	0.899	.0262	0.392

NOTE: χ^2 is calculated for the deviation from unity (see footnote, table 2).

The *Neurospora* data need a note of explanation: It has been suggested that if the two center nuclei were occasionally to exchange places, following the second meiotic division, the effect would be indistinguishable from that of simultaneous crossing over on both sides of the attachment when this is used as a marker. Such a slipping of the nuclei, if it occurred, would therefore tend to increase the coincidence between the *g* attachment and attachment *c* regions. When region + *g* is compared with region attachment *c*, any such spurious crossovers in the latter would be independent of true crossovers in the former and would tend to make the coincidence

nearer unity than it would otherwise be. When regions + *g* and *c p* are compared, slipping of the nuclei could not affect the coincidence, since the attachment is not used as a marker. In all of these three comparisons coincidence is greater than one, and the degree of statistical significance is extremely high. It is of interest to note that the accuracy of scoring is increased in *Neurospora* by the facts (a) that each of the four products of meiosis is represented by two identical nuclei which may be used as checks on each other, and (b) that in an ascus from a heterozygote the two allelomorphs must each occur twice in the four products of meiosis.

TABLE 7
*Crossover and chiasma coincidence across the attachment in
Drosophila and Neurospora.*

REGIONS	a	b	x	n	c	V(c)	χ^2	P
Drosophila (chromosome III)								
<i>D-p^p-e^e</i>	88	430	29	2191	1.679	0.062	7.480	< .01
Neurospora (chromosome I)								
+ - <i>g-sfa</i>	40	30	6	422	2.110	0.516	2.386	
<i>g-sfa-c</i>	30	32	11	422	4.835	1.096	13.422	< .001
<i>sfa-c-p</i>	32	82	8	422	1.287	0.141	0.582	
+ - <i>g, sfa-c</i>	40	32	11	422	3.627	0.651	10.602	.001
<i>g-sfa, c-p</i>	30	82	5	422	0.858	0.115	0.176	
+ - <i>g, c-p</i>	40	32	28	422	3.602	0.172	39.467	very small

NOTE: *sfa* denotes the spindle fiber attachment, which has been used as a marker. *a*, *b*, *x*, *n*, *c*, and *V(c)* are as in the other tables except that for the *Drosophila* data they are in terms of crossovers and chromatids, whereas for the *Neurospora* data they are, as for *Trillium*, in terms of chiasmata and bivalents. χ^2 is calculated for the deviation from unity (see footnote, table 2). The *Drosophila* data are from BRIDGES and MORGAN 1923, p. 129, table 87; the *Neurospora* data are from LINDEGREN and LINDEGREN 1937, p. 109, table 2.

It should be noted that *Diachaete* is associated with an inversion which might affect both the pairing relationships and the recoverable crossovers.

We may therefore say that genetic data agree with the cytological observations in *Trillium* that coincidence across the attachment can be greater than one. This implies a certain heterogeneity in the data, the biological cause of which is not known. KIKKAWA (1935) has suggested that it is the result of variability in pairing, and although this is a difficult hypothesis to test, it at least provides a mechanism which explains the data and which is easy to visualize.

It is interesting to note that the slides having the higher chiasma frequency and an average of 72.2 percent chiasma formation in the regions adjacent to the attachment (as compared to 51.4 percent in the lower chiasma frequency material) appear to have a lower coincidence across the attachment. Thus, in *Trillium* the greater the regularity with which

chiasmata are formed in these regions, the less evidence there is of heterogeneity. In the arms no such relationship is observed, but in this case there are three possible variables indistinguishable from one another which would affect the coincidence. These are: (1) heterogeneity within the regions compared, (2) heterogeneity between the regions of an arm which have been grouped to give an estimate of coincidence for that arm, and (3) interference. The first two would, of course, tend to increase the coincidence value and the third to decrease it.

As in *Drosophila*, interference disappears as the distance between the regions is increased. In *Trillium* statistically significant evidence of interference is found when the regions are separated by as much as two microns. This is a minimum estimate of the distance over which interference can act, since it may be effective at greater distances but be obscured by heterogeneity both within and between the regions grouped to give these estimates. That there is no significant deviation of coincidence from unity when the distance between the regions is great, is in agreement with STEVENS' (1936) interpretation of WEINSTEIN'S (1918) *Drosophila* data.

In making this comparison of cytological with genetic coincidence, it has been assumed that chiasma movement and loss prior to the time of observation is negligible. A similar assumption is involved in the analysis of HALDANE (1931), who noted that reduction of chiasma frequency through terminalization could effect a reduction in variance, from which he determined the existence of cytological interference. He considered, however, that "this would hardly account for the very small variance found." There is no indication of chiasma movement in *Trillium*. Evidence that there is no movement is found in the analysis of MATHER (1940) and in the observation that different types of chiasma pairs observed by HUSKINS and NEWCOMBE (1941) tend to be of different lengths, whereas were there an appreciable rearrangement due to repulsion between chiasmata, all the simpler types would tend to be of the same length.

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SUMMARY

A detailed study of interference (and coincidence) between chiasmata has been made in *Trillium erectum*.

Chiasma coincidence is found to be significantly greater than unity across the attachment; published genetic data are analysed showing that this is also the case in the third chromosome of *Drosophila* and the first

chromosome of *Neurospora*. This is assumed to be the result of heterogeneity, the biological cause of which is not clearly indicated.

An increase in total chiasma frequency appears to be accompanied by increased coincidence within the arms and decreased coincidence across the attachment, although the observed differences are not quite within the limits of statistical significance.

Coincidence within the arms increases with the distance between chiasmata and, as reported in *Drosophila*, reaches a value not differing significantly from unity.

The observed similarities to genetic data on interference add further evidence favouring the assumption that all chiasmata are concomitants of crossing over.

LITERATURE CITED

- BELLING, J., 1929 Nodes and internodes of trivalents of *Hyacinthus*. Univ. of Calif. Pub. Bot. **14** (18): 379-388.
- BRIDGES, C. B., 1929 Variation in crossing-over in relation to age of female in *Drosophila melanogaster*. Pub. Carnegie Instn. **399**: 63-89.
- BRIDGES, C. B., and MORGAN, T. H., 1923 The third chromosome group of mutant characters of *Drosophila melanogaster*. Pub. Carnegie Instn. **327**: 21-251.
- DARLINGTON, C. D., 1930 A cytological demonstration of "genetic" crossing-over. Proc. Roy. Soc. B. **107**: 50-59.
- 1937 Recent Advances in Cytology. 2nd ed. London: Churchill and Co.
- HALDANE, J. B. S., 1931 The cytological basis of genetical interference. Cytologia **3**: 54-65.
- HUSKINS, C. L., and NEWCOMBE, H. B. 1941 An analysis of chiasma pairs showing chromatid interference in *Trillium erectum*. Genetics **26**: 101-127.
- KIKKAWA, H., 1935 Biological significance of coincidence in crossing-over. Japanese J. Genet. **11**: 51-59.
- LINDEGREN, CARL C., and LINDEGREN, G., 1937 Non-random crossing-over in *Neurospora*. J. Hered. **28**: 105-113.
- MATHER, K., 1938 Crossing-over. Biol. Rev. **13**: 252-292.
- 1940 The determination of position in crossing-over. J. Genet. **39**: 205-223.
- MULLER, H. J., 1925 The regionally differential effect of x-rays on crossing-over in autosomes of *Drosophila*. Genetics **10**: 470-507.
- REDFIELD, H., 1930 Crossing-over in the third chromosomes of triploids of *Drosophila melanogaster*. Genetics **15**: 205-252.
- STEVENS, W. L., 1936 The analysis of interference. J. Genet. **32**: 51-64.
- WEINSTEIN, A., 1918 Coincidence of Crossing-over in *Drosophila melanogaster* (*ampelophila*). Genetics **3**: 135-172.