Meiosis in *Paris*
I. Mechanism of Chiasma Formation

By

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(With 21 Text-figures)

Contents

Introduction

One of the most interesting problems in cytology during the last decade was apparently to find the cytological feature of the genetical crossing over. Since it has been advocated by JANSSENS (1909, 1924), BELLING (1929, 1931, 1933), DARLINGTON (1930, 1937), MAEDA (1930) and others that the cytological chiasmata must be accompanied by genetical crossing over, many followers, especially DARLINGTON himself and his school, have intensely endeavored to make out the cytological behavior of chiasmata in lieu of genetical behavior of crossing over (cf. DARLINGTON 1937, SHARP 1934). Through innumerable investigations on this line there were found certain parallelisms between these two phenomena genetical and cytological (cf. DARLINGTON l. c., MATHER 1938). Despite the present status that the chiasmatype theory or one-plane theory of chiasma formation, just mentioned above, seems to be accepted generally by the overwhelming majority of

the recent cytologists as well as geneticists, the theory rests as yet fundamentally on the unproved prime presumptions that the kinetochores and four daughter chromatids separate invariably reductionally, in strict genetical sense, at diplotene and that the cytological chiasmata do result from genetical crossing over. Therefore the concomitant phenomena in behavior of chiasma formation and crossing over can not be regarded as convincing to prove that chiasma is formed as a consequence of crossing over. In fact most of the so-called evidences for one-plane theory are shown to be explicable alternatively on the basis of opposing two-plane theory of chiasma formation (cf. MATSUURA 1937a, c, 1938, 1940, 1941b, MATSUURA and HAGA 1942).

According to the classical two-plane theory chiasma is formed by the alternate two-by-two opening out of the four chromatids, equational as well as reductional opening out having occurred at diplotene. In this manner of formation chismata have no relation, at least in their origin, to the genetical crossing over. This represents the view held by many classical cytologists (cf. SHARP l. c.).

Cytological and genetical evidence is as yet not sufficient to decide which of the above two interpretations expresses the real event. Some phenomena have been held to conform better to the one-plane theory and others to the two-plane theory, but none of them can be regarded as decisive (cf. SHARP l. c.). However, a series of recent investigations by MATSUURA (l. c.) have rendered doubtful the validity of the one-plane theory, which has been expanded extensively on the basis of unproved conjectures. The chief aim of the present study is to reinvestigate the causal mechanisms involved in chiasma formation from the new standpoint on the basis of the neo-two-plane theory of bivalent constitution (MATSUURA 1937a, c, 1938). Accordingly it will be well to quote here briefly the principles of this new theory. The main principles are as follows: "(i) the mode of two-by-two opening of the four daughter chromatids (excluding their kinetochores) at diplotene is at random, no difference in behavior existing between the sister and non-sister strands, (ii) the two-by-two assortment of the four daughter kinetochores at the first metaphase follows chance too, no difference in behavior existing between the sister and the non-sister ones, and (iii) the mode of chromatid opening is entirely independent from that of kinetochore separation, every free combination of these two being possible" (MATSUURA 1938, p. 78). These principles are, in contradistinction to those of the previous theories, those established on the ground of the extensive good statistics in an excellent cytological material *Trillium kamtschaticum*.

The most important implication, in relation to the present study, of the above principles is that chromosomes as well as kinetochores, which were synapsed effectively at the time of pairing, separate either equationally or reductionally, following the law of chance, at the time of separation. Thus equational and reductional opening out, at diplotene, of a synapsed arm pair is to occur with the ratio 2 equational: 1 reductional, three different modes, two different equational and one reductional, being possible with equal chance. This is proved in several ways in *Trillium kamtschaticum* and *Paris verticillata*. For example, though it represents rather specific case, the ratio between the frequencies of the two modes of opening out was found to be 667 equational: 333 reductional in a total of 1000 heteromorphic arm pairs of *Paris verticillata* (a part of the present study quoted in MATSUURA 1938). Consequently, it follows that the chiasmata can be formed by the meeting of the diplotene loops which have been developed differently in their modes of opening out. In this manner of formation chiasma is formed without any preceding crossing over as maintained in the classical two-plane theory. Basing on this fundamental consideration, a consistent interpretation was given successfully by the present study for a series of problems concerning the cause and consequence of chiasma formation.

The present study has been completed under the supervision of Professor HAJIME MATSUURA, to whom the writer wishes to express cordial thanks for his invaluable suggestions and criticisms. The writer is also grateful to his colleagues for their valuable discussing in the course of this work. A financial aid was received for the present study from the Scientific Research Fund of the Department of Education. It is the writer's pleasant duty to express here his thanks to this foundation.

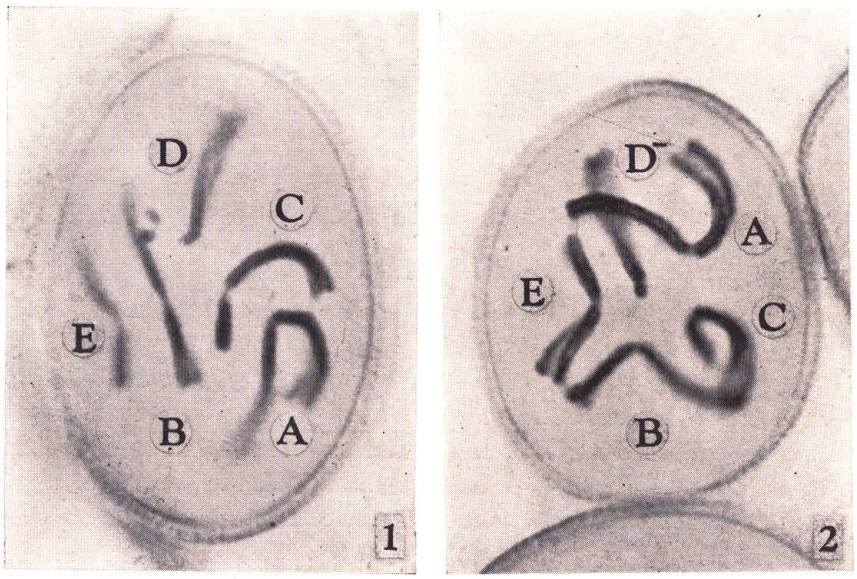
Material and Methods

Data for analyses were accumulated by observations on meiosis in pollen mother-cells of *Paris verticillata* MARSCHALL VON BIEBERSTEIN[1]. This plant is excellently favorable for such cytological study as is the case in *Trillium kamtschaticum* PALLAS which was employed widely by MATSUURA (l. c.) in his extensive series of investigations. The advantages consist in (1) large size and small number, only five pairs, of chromosomes, (2) appropriate length differences between the chromosomes and even between separate arms, (3) ease in identifying the individual chromosomes at

1) Formerly synonyms *P. quadrifolia* L. var. *obovata* REGEL. et TIL. or *P. hexaphylla* CHAM. were used.

meiosis by their length and the position of kinetochore, (4) appropriate chiasma frequency which renders reliable the recording of chiasma frequency, and (5) absence of so-called terminalization or other movement of chiasmata (Figs. 1–2 and 8–9).

A diploid karyotype 2n–III from one and the same population was employed exclusively in the present study. This form is identical with the karyotype CCDD⁻ in the writer's previous papers (HAGA 1934, 1937). Chromosome designations A, B, C, D and E were used unaltered in the present paper. In all the chromosome pairs of this karyotype, except chromosome pair DD⁻, partners are morphologically indistinguishable. One of D type chromosomes is normally satellited (chromosome D) and the other is completely deprived of its entire satellite (chromosome D⁻). Irrespective of the presence or absence of the satellite, D type chromosomes represent the sole nucleolar chromosome in the complement, all others being non-nucleolar under the natural condition (HAGA 1942). In accord with this heterozygosity the present karyotype produces two kinds of pollen-grains with regard to the D type chromosomes. Actually 537 pollen-grains containing chromosome D and 533 pollen-grains containing chromosome D⁻, nearly exactly 1:1 ratio, were observed in a total of 1070 pollen-grains at primary mitosis (Figs. 1–2).



Figs. 1–2. Two kinds of pollen-grains produced by karyotype 2n-III. Metaphase of the primary mitosis. **1**, a pollen-grain containing chromosome D. **2**, a pollen-grain containing chromosome D⁻. ×2050.

The mean length of each chromosome obtained by averaging eight complete root-tip metaphases is presented in Table 1. Length of the chromosomes in somatic mitosis is variable between nuclei even within the same individual. However their relative length within a nucleus remains highly constant (cf. HAGA 1934, 1937). Similar variation also occurs in the meiotic chromosomes, however variation within an individual or within a population is negligible, in so far as the individual or population undergoes meiosis within a certain limit of environmental conditions (cf. MATSUURA 1935, 1937b, MATSUURA and HAGA 1940). On these grounds, in the present study the relative length measured in root-tip mitosis was used as the measure of lengths.

Table 1. Length of the somatic chromosomes (HAGA 1937).

Chromosome	Length in micron				Relative length (%)
	Long arm	Short arm	Satellite	Total	
A	12.8	12.5	—	25.3	27.6
B	11.1	7.9	—	19.0	20.7
C	12.7	4.7	—	17.4	19.0
D	13.2	1.0	1.9	16.1	17.6
E	8.1	5.7	—	13.8	15.1
Total				91.6	100.0

Meiosis in pollen mother-cells was observed exclusively with the iron aceto-carmine smear and mitosis in pollen-grains with the permanent smear fixed with LA COUR 2BE and stained by NEWTON's gentian-violet-iodine method. All drawings were made with an ABBÉ camera lucida using a LEITZ oil immersion objective n.A. 1.3 and an LEITZ periplan eye piece ×15, giving a magnification of ×1750 diameters. In reproduction they were reduced to the scale indicated.

Data and Interpretations

Before describing in detail, general remarks will be noted below for convenience' sake. Firstly, as in the case of *Trillium kamtschaticum*, kinetochores of the homologous chromosomes generally remain synapsed until late first metaphase (cf. MATSUURA 1941a). On account of this circumstances, a synapsed kinetochore pair appears as if it were a chiasma node, but was excluded intentionally from the recording of chiasma frequency. The same method has been already adopted in *Trillium*

kamtschaticum (MATSUURA 1937a, MATSUURA and HAGA 1942). This is the essential difference from the method applied in general by the previous workers who have paid no attention to the behavior of kinetochores even in the case of organisms with so-called localized chiasmata. The distinction of these two interstitial junctions, which are of entirely different nature, is not difficult by virtue of the differences in chromosome lengths and of the known position of kinetochores and further, in favorable cases, of the faintly stained conical protrusion of kinetochores. The case where a kinetochore pair is already separated before metaphase must be dealt with separately from the above case.

Secondly, terminal chiasmata, which are believed on the chiasmatype theory to be derived from the so-called terminalization of interstitial chiasmata, were recorded and treated separately from interstitial chiasmata. In the present paper the term chiasma, with or without the adjective interstitial, is adopted for interstitial ones, the terminal chiasma being distinguished from the interstitial ones by always adjoining the ajective terminal. They were represented, respectively, with the abbreviation X and TX in singular and Xta and TXta in plural.

Finally, it must be remarked that recently two kinds of chiasmata were distinguished in *Trillium kamtschaticum,* that is, primary and secondary (MATSUURA 1941b). In the present study only primary chiasmata are dealt with, since secondary ones are not detectable in usual aceto-carmine preparations. The primary chiasmata are determined, as will be shown later, by the main opening out in pairs of two chromatids of the paired chromosomes, and the secondary chiasmata are developed subordinately to the primary main opening out. So that the present study is confined only to elucidate in what manner the primary main opening out, accordingly primary chiasmata, are developed[1].

1. Pairing and separation of a heteromorphic chromosome pair[2]

Heteromorphic chromosome pair DD$^-$ forms a bivalent as regularly as the remaining homomorphic pairs at meiosis. This particular pair shows unmistakably two different configurations at first metaphase in respect to its heteromorphic short arm pair. One is the configuration showing a single comparably large protrusion from the synapsed kinetochores. The

1) cf. foot-note 2) in p. 80.

2) The data in this paragraph were quoted and discussed by MATSUURA (1938). Nothing is altered from his original interpretations.

other is one having two protrusions, instead of one, of which one being large and the other markedly small in size (Figs. 3, 8–9 and 15–17). The former was termed as "closed arm" and the latter as "open arm" configuration respectively by MATSUURA (1938). Larger protrusion in the latter configuration represents obviously the satellited short arm of chromosome D, and the small one the short arm of non-satellited chromosome D^-. The distinction of these two configurations consists in the difference in the modes of diplotene opening out. The closed arm or equational configuration will arise when four short arm chromatids open out equationally, and

Table 2. Frequency of equational and reductional first metaphase configurations of the heteromorphic short arm pairs of bivalent DD^-.

Year	E	R	Total	E : R (%)
(1937)	394	206	600	65.7 : 34.3
(1938)	273	127	400	68.3 : 31.8
Total	667	333	1000	66.7 : 33.3

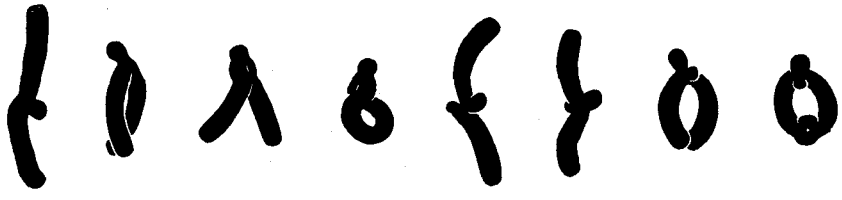


Fig. 3. Metaphase bivalents consisting of chromosome D and D^-. Four bivalents on the left are those of closed arm or equational configuration and the remaining four on the right those of open arm or reductional one. ×1050.

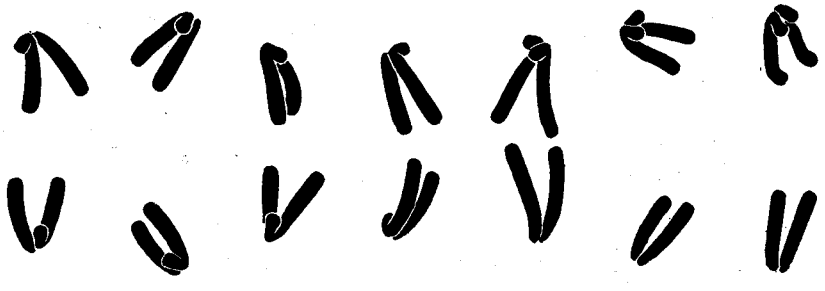
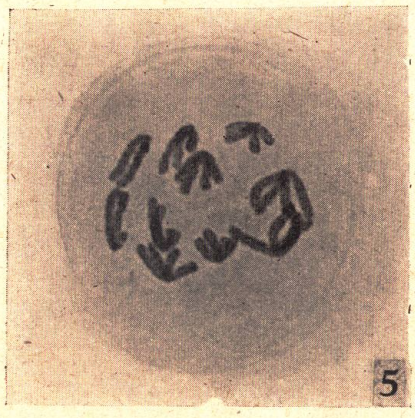


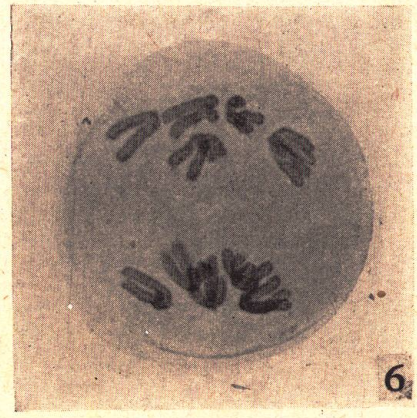
Fig. 4. First anaphase separations of the heteromorphic bivalent DD^-. With regard to the heteromorphic short arms the separation is equational in the four pairs of half-bivalents on the left and reductional in three pairs on the right. ×1050.

the open arm or reductional one will result when the mode of opening out is reductional (Fig. 7). If this is true, then the former must appear twice as frequent as the latter in accordance with the expectation from the principle of the neo-two-plane theory. In fact, this is the case, the ratio 667E:333R[1] being obtained in a total of 1000 short arm pairs (Table 2).

On the chiasmatype theory the metaphase configuration, which is interpreted above as the result of equational diplotene opening out, will be explained as postulating a chiasma or number of chiasmata formed in the equal segment short arm between the kinetochore and the differential segment satellite and terminalized to the distal end of the equal segment (cf. HUSKINS and SPIER 1934, KOLLER 1936, 1938a, KOLLER and DARLINGTON 1934, etc.). However, such an interpretation can be hardly applicable in the present case as pointed out below.

At first anaphase two different modes of separation are easily distinguished with regard to the heteromorphic short arm pairs, that is reductional and equational separations, according to whether the two satellited chromatids pass to the same pole or separate to opposite poles (Figs. 4–6). Observed ratios between the frequencies of these two modes of the first anaphase separations are presented in Table 3. In grand total





Figs. 5–6. First anaphase. Separation of the heteromorphic bivalent DD$^-$ is seen at the left-most in each photo. **5**, satellited chromatids are separating to the opposite poles, the short arm separation being thus equational. **6**, both satellited chromatids are passing to the same lower pole, the short arm separation being thus reductional. ×760.

1) Throughout the present paper the abbreviations E and R are used respectively to represent equational and reductional mode of diplotene opening out or of anaphase separation.

of 2000 first anaphases the ratio was 82.3E : 17.8R in percentage.

On the chiasmatype theory one chiasma represents one genetical crossing over and the kinetochores separate always reductionally at first anaphase. If this is true, the proportion of the equational first anaphase separation of the heteromorphic short arm pair is determined by the number of chiasmata formed between the satellite and the kinetochore. This relation is expressed by MATHER (1935c) as

$$E_n=\frac{2}{3}\left[1-\left(-\frac{1}{2}\right)^n\right],$$

where E_n denotes the proportion in the case involving n chiasmata. According to this formula, maximum proportion, 1 or 100 in percentage, is attained with the formation of one chiasma. The minimum proportion is 0.5 or 50 per cent and is attained with the formation of two chiasmata, excepting the case of non-formation of the chiasma where the proportion is naturally 0. Then equational first anaphase separation is expected to occur with the frequency within the range 100–50 per cent of the frequency of the equational configuration at first metaphase, that is, to occur with the frequency within the range 66.7–33.3 per cent. But in reality we found the frequency of 82.3 per cent at first anaphase, at least, 15.6 per cent being in excess of the expected maximum. On the other hand, if the chiasmata are the direct consequence of the genetical crossing over, the value of recombination of the two definite loci is

$$p=\frac{1}{2}(1-a),$$

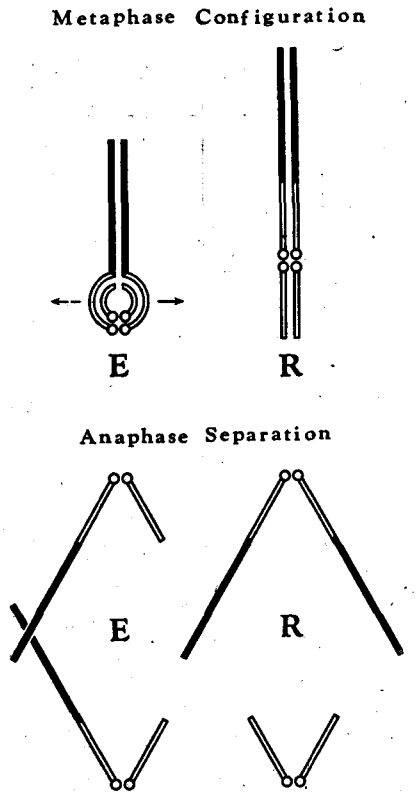


Fig. 7. Diagrammatic representation of the metaphase configurations and anaphase separations of the heteromorphic short arm pair of bivalent DD⁻. Circles represent daughter kinetochores. Equal segment, the short arm proper, is shown in blank and the differential segment, the satellite, in black. Long arm pairs are not shown. The arrows indicate the direction of the repulsion force acting between short arms and interfering with the random assortment of the daughter kinetochores.

Table 3. Frequency of equational and reductional first anaphase separations of the heteromorphic short arm pairs of bivalent DD⁻.

Year	E	R	Total	E : R (%)
(1935)	213	40	253	84.2 : 15.8
(1937)	611	136	747	81.8 : 18.2
(1938)	821	179	1000	82.1 : 17.9
Total	1645	355	2000	82.3 : 17.8

where p stands for recombination value and a for frequency of non-formation of the chiasma in the region under consideration (MATHER 1938). Of course here the sum of the frequencies of formation of 0, 1, 2 etc. chiasmata in the chromosome region under consideration is 1. Then, if kinetochores separate always reductionally, the frequency of equational first anaphase separation represents the bulk proportion of recombination between kinetochore and the satellite. But the proportion of the equational separation does not correspond to the entire proportion of the real recombination, since certain cases, such as four strand double crossing over, are cytologically classified to the reductional separation. Hence the real recombination must be greater than 82.3/2, that is, more than half the frequency of equational first anaphase separation. Therefore the frequency of equational configuration at first metaphase is to be at the minimum 82.3 per cent and that of reductional one must be at the maximum 17.8 per cent. This expectation also conflicts with the real frequencies 66.7E:33.3R. MATHER's formulas are justifiable only when there exists no chromatid intereference in chiasma formation. However, HUSKINS and NEWCOMBE (1941) have inferred the presence of chromatid interference in *Trillium erectum*, which leads to the excess in the compensating chiasma pairs. If so, though the method of the latter workers is disputable, the discrepancy between the expected and observed frequencies becomes much serious. Thus chiasmatype theory fails completely to account for the present observations.

According to the principles of the neo-two-plane theory, the mode of first anaphase separation of the bivalents is determined solely by that of the kinetochores. The latter occurs either equationally or reductionally by chance, resulting the ratio 2E:1R or 66.7E:33.3R in percentage. However the actual raito was 82.3E:17.8R, much deviating from the ratio on the randomness of occurrence of the two modes of separation. The divergence of the actual ratio from the expectation will be comprehensible with the following interpretation. In the closed arm or equational metaphase con-

figuration a specific very small loop is obligatorily formed just next to the kinetochores which remain paired until the first anaphase is commenced. As the loop is exceedingly small, the repulsion force acting between the short arms will remain unsatisfied until the anaphase separation is completed. This repulsion force will act to expand the loop outwards, thus interfering with the random assortment in a pair of two of the four daughter kinetochores. The effect of interference necessarily acts to separate the four daughter kinetochores in the same mode of separation with which the short arm chromatids opened out at diplotene, that is, to separate equationally (Fig. 7). Thus the interference in this manner will lead to an excess in the proportion of equational separations. Generally saying, the interfering power of such a loop is reasonably considered to become stronger as the loop decreases its length. The chiasma loop next to the kinetochore in the long arm pair of this bivalent will interfere with the kinetochore separation in the same manner. Indeed the loop of secondary chiasma just next the kinetochore, if it present itself, would act likewise as the closed arm configuration. But the interference of the ordinary chiasma loop will be negligible since its length is incomparably larger than the specific loop of the closed arm configuration. Then it follows that the proportion of the equational first anaphase separations at minimum and maximum interference of the closed arm loop, setting aside the effect of the secondary chiasma loop in the long arm pair, will be 66.7 and 88.9 per cent respectively. The calculation of these values is given in Table 4.

Table 4. Ratio between proportions of equational and reductional first anaphase separations at minimum and maximum interference of the closed arm loop.

Configuration ratio (%)	66.7E : 33.3R		In total
Separation ratio (%)			
Minimum interference	44.4E : 22.2R	22.2E : 11.1R	66.7E : 33.3R
Maximum interference	66.7E : 0.0R	22.2E : 11.1R	88.9E : 11.1R

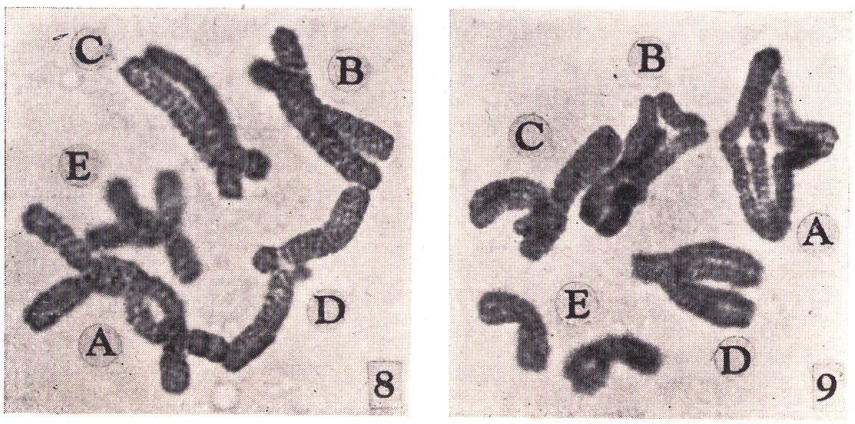
These two pairs of proportions calculated represent the two extremes. Consequently it may be safely concluded that the proportion of the equational separations of the heteromorphic pairs must fall between those two extreme values in the cases where the kinetochores really synapse and their separation is posterior to that of the remaining chromosome portions as in the present instance. The proportions in individual cases are, of course, variable according to the length of the equal segment between the kineto-

chore and the differential segment. Basing on these considerations, the grade of interference of the closed arm loop in the present case will be calculated approximately as 100 (82.25−66.67)/22.22=70.12 per cent. Generally speaking, the diversity in this value will be one of the causes which lead to the diversity in the E:R ratios in the first anaphase separations of the heteromorphic pairs in different organisms. Further discussion on this problem will be made later in relation to the different modes of chromosome pairing.

Concluding the present item it must be emphasized that the present observation is at marked variance with the assumption of the chiasmatype theory but in complete conformity with the principle of the neo-two-plane theory. Consequently, it is justified to adopt the principles of the latter theory throughout in the following analyses.

2. *Frequency of interstitial chiasmata in relation to chromosome length*

Individual chromosome pairs of *Paris verticillata* are identifiable in meiosis as easily as in somatic mitosis (Figs. 8–9). This made it possible to record the chiasma frequencies of the individual chromosome pairs. Chiasma frequency of each separate arm pair of the individual bivalents



Figs. 8–9. First metaphase. **8**, 5Xta=1($A\frac{1}{2}$)+2($A\frac{1}{2}$)+1(B_s)+1(E_1). Note the small interstitial loop in the arm pair with 2Xta of bivalent A. Configuration of D_s pair is reductional. **9**, 3Xta=1($A\frac{1}{2}$)+1($A\frac{1}{2}$)+1(C_1) and 1TX(B_s). Note the separated kinetochores of bivalent A, bridge and fragment visible in B_1 pair and the univalent pair of chromosome E. Configuration of D_s pair is equational. See foot-note to Table 7. ×1460.

was recorded in 600 and 400 complete first metaphase plates respectively in the years 1937 and 1938. Kinetochores, which maintain their prophase pairing until the first anaphase, were excluded from the statistics of the chiasma frequencies. The terminal chiasmata were treated separately from the interstitial ones. Number of interstitial chiasmata per nucleus ranges from 0 to 9, and that of terminal ones per nucleus from 0 to 3 (Table 5). The average number of interstitial chiasmata per nucleus was 2.94 in 1937 and 3.94 in 1938, that of terminal chiasmata being 0.34 and 0.21 respectively (Table 5). Frequencies of various configurations taken by individual arm pairs are given in Table 7.

Table 5. Frequency of nuclei with different numbers of interstitial and terminal chiasmata.

(1937)

Xta per nucleus		0	1	2	3	4	5	6	7	8	9	Total
TXta per nucleus	0	15	51	108	93	92	52	15	5	1	1	433
	1	4	27	33	27	31	8	4	—	—	—	134
	2	1	5	9	6	7	1	—	—	—	—	29
	3	—	—	—	3	1	—	—	—	—	—	4
Total		20	83	150	129	131	61	19	5	1	1	600

In total: 600 nuclei, 1765 Xta, 204 TXta.
Per nucleus: 2.94 Xta, 0.34 TXta.

(1938)

Xta per nucleus		0	1	2	3	4	5	6	7	8	9	Total
TXta per nucleus	0	4	15	35	73	94	59	33	14	3	—	330
	1	—	2	6	14	12	14	8	—	2	—	58
	2	—	1	2	3	3	3	—	—	—	—	12
	3	—	—	—	—	—	—	—	—	—	—	0
Total		4	18	43	90	109	76	41	14	5	0	400

In total: 400 nuclei, 1574 Xta, 82 TXta.
Per nucleus: 3.94 Xta, 0.21 TXta.

Through the present statistics it has been revealed rather astonishingly that the chiasma frequency is not proportional to the entire length of chromosome (Table 6). On the contrary it showed significant association with the length of separate arms, the frequency being increased with the increase in arm length (Table 7). As repeatedly mentioned, in the present material, kinetochores of the homologous chromosomes maintain their prophase pairing until first anaphase is commenced. So that the paired kinetochores act as a fixed point in opening out at diplotene, dividing the chromo-

Table 6. Frequency of interstitial and terminal chiasmata in individual chromosome pairs.

Chromosome Relative length (%)		E 15.1	D 17.6	C 19.0	B 20.7	A 27.6	Total 100.0
(1937)	Xta	189	367	324	310	575	1765
	TXta	32	36	37	47	52	204
	Total	221	403	361	357	627	1969
(1938)	Xta	203	323	310	276	462	1574
	TXta	13	18	8	26	17	82
	Total	216	341	318	302	479	1656

Table 7. Frequency of arm pairs with different numbers of interstitial and terminal chiasmata.*

(1937)

Chromosome arm Relative length (%)		C_s 6.0	E_s 7.2	B_s 10.0	E_l 10.3	B_l 14.1	$A_{\frac{1}{2}}$ 16.0	C_l 16.1	D_l 16.7	Total 96.4
0X+0TX		579	553	457	427	392	306.5	277	225	
0X+1TX		3	7	23	24	19	16.5	25	19	
1X+0TX		18	40	119	148	184	257.0	281	328	
1X+1TX		—	—	1	1	4	9.5	9	17	
2Xta+0TX		—	—	—	—	1	10.5	8	11	
2Xta+1TX		—	—	—	—	—	—	—	—	
Total	Arm pairs	600	600	600	600	600	600.0	600	600	4800
	Xta	18	40	120	149	190	287.5	306	367	1477.5
	TXta	3	7	24	25	23	26.0	34	36	178.0

(1938)

Chromosome arm Relative length (%)		C_s 6.0	E_s 7.2	B_s 10.0	E_l 10.3	B_l 14.1	$A_{\frac{1}{2}}$ 16.0	C_l 16.1	D_l 16.7	Total 96.4
0X+0TX		383	349	387	237	221	184.5	143	97	
0X+1TX		1	3	10	8	9	3.0	1	6	
1X+0TX		16	48	103	153	160	188.5	214	260	
1X+1TX		—	—	—	2	7	5.5	4	11	
2Xta+0TX		—	—	—	—	3	18.5	36	25	
2Xta+1TX		—	—	—	—	—	—	2	1	
Total	Arm pairs	400	400	400	400	400	400.0	400	400	3200
	Xta	16	48	103	155	173	231.0	294	323	1342.0
	TXta	1	3	10	10	16	8.5	7	18	73.5

* Subscript 1 and s denote long and short arm respectively. Subscript $\frac{1}{2}$ annexed to A indicates that length and chiasma frequency of an arm pair of this bivalent were given dividing by 2 the entire chromosome length and the total chiasma frequency respectively, since in this bivalent the two arms are indistinguishable from each other by their length. The same denotations are used throughout the present paper. Relative lengths of D_s and D_t, satellite, are 1.3 and 2.4 respectively.

somes into two independent portions. Thus the two arms of a chromosome are completely independent in chiasma formation. This characteristic nature of the paired kinetochores alone must be responsible for the observed fact that the chiasma frequency is proportional to the arm length but not to the entire chromosome length.

It will be seen in the data presented in Table 7 that there are two distinct classes in arm lengths as to the number of interstitial chiasmata formed in them. One is the class in which one chiasma is formed, but never two chiasmata, and the other represents the class in which as many as two chiasmata can be formed, but never more than two chiasmata. To the former class belong the arms C_s, E_s, B_s and E_1, and to the latter B_1, A_1, C_1 and D_1. These two length classes will be referred to hereinafter as one-chiasma-length and two-chiasma-length class respectively. As is evident in the comparison of the two data in Table 7, demarcation between these two classes and the maximum number of chiasmata to be formed in each class are not altered by the increased mean chiasma frequency. The same fact is quite convincing in the results of an experimental study in *Trillium kamtschaticum* (MATSUURA and HAGA 1942). The same two length classes as in *Paris verticillata* are distinguished in *Trillium kamtschaticum*, each class comprising the arms closely similar to those of *Paris verticillata*. Under the influence of high temperature the chiasma frequencies were markedly enhanced, in extreme case the frequency attaining to 14.5 times the frequency in control materials. Even in those extreme cases there was never noticed the shifting of the demarcation between the two length classes and in the maximum number of interstitial chiasmata formed in each length class, in one-chiasma-length class never more than one interstitial chiasma being formed and in the two-chiasma-length class never more than two interstitial chiasmata (MATSUURA and HAGA l. c.). Therefore the maximum number of chiasmata to be formed in an arm represents a constant inherent to the individual arm length.

The above findings are very important in revealing that increase in chiasma frequency is accompanied by the increase in chance with which chiasmata are formed but not by the rising of the maximum number of chiasmata which are formed in an arm pair. Accordingly it follows necessarily that there exists a definite minimum length in opening out and that this minimum length remains constant even under the different environmental conditions.

Initiation of an opening out can arise at any possible position in the paired chromosomes. The opening out of the initiating point will suppress

in time and space the occurring of another independent opening out in its neighborhood within certain definite distance from it. In other words the first step of an opening out will appear as a block of splitting which was completed suddenly but not developed gradually. Opening out in this fashion will be termed as the primary opening out and its effect in suppressing the occurrence of another opening out in the adjacent region as the primary interference. Constancy of the distance of the primary interference must be responsible for the constancy of the length classes as to the maximum number of interstitial chiasmata to be formed in them. Otherwise no clue will be gained to account for the observed constancy of the length classes. As will be natural, the primary interference can not be effective across the paired kinetochore, through a chiasma and beyond the free end of an arm pair (Fig. 10a). Consequently, distance of the primary interference to one side of the initiating point is to represent the unit length in the primary opening out. On the contrary, if we assume the sum of the distances at either sides as the unit length, there arises the conflict that another opening out can occur within the range of the primary interference when the initiating point is located near the kinetochore or the distal end. Empirically this unit length in the primary opening out is not equal in the region adjoining to the paired kinetochores and in the regions apart from the kinetochores with certain distance. The unit length in the former region is represented with k and that in the latter with l, the fact that $k<l$ being apparently due to the physical nature of the paired kinetochores. The values of the constants k and l are supposed to be universal between arms of different length, since there seems to be no reason to assume the specialization in each different length.

After the completion of the primary opening out, unopened regions adjoining to the primary opening out will be forced to open out in the same fashion with which the primary opening out has been completed. This consideration is substantiated with the fact that the closed arm loop of the heteromorphic bivalent DD^- interferes with the separation mode of the paired kinetochores. Interference in this fashion may be termed as secondary interference in opening out, and the opening out developed under its influence as the secondary opening out. Secondary opening out will proceed gradually at both sides of the primary opening out until it meets with another opening out which developed from the second initiating point in the same arm or until the development is cancelled at the distal end or arrested by the paired kinetochores. Namely, the distance of the secondary interference is variable, being functional to the time during which it is

allowed to act.

At any rate, if the modes of opening out are different between the two openings out which meet with each other, there results a chiasma. On the contrary, if the modes of opening out are identical between the two openings out, no chiasma is formed. If the entire length of an arm pair is opened out under the influence of the primary or both of the primary and secondary interferences of a single initiating point, obviously, there occurs no chiasma formation. In view of the foregoing considerations it is evident that three factors are responsible for determining the chiasma frequency: (1) primary interference which is represented by k and l, the values of these two being universal between arms of different lengths and constant even under altered environmental conditions, (2) secondary interference, its distance being functional to the time during which it is allowed to act, and (3) difference and sameness in the modes of opening out between the openings out which meet with each other in an arm pair, three modes, two different equational and one reductional, being possible with equal chance.

Now suppose an arm pair shorter than k, that is $k>L$, where L stands for arm length. In this length entire length of the arm is overrum by a single primary opening out, whatever may be the position of the initiation in opening out (cf. Fig. 10b). Accordingly, no chiasma is formed as far as the length does not exceed the limit that $L=k$. In the next length class, one-chiasma-length class, where $k<L<(k+l)$, two main factors are distin-

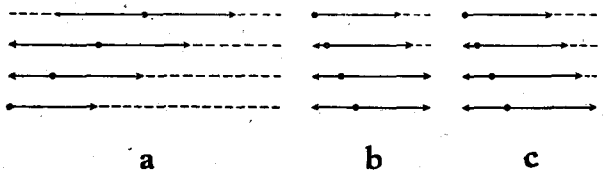


Fig. 10. Diagrammatic representation of the primary opening out in relation to arm length. Initiating point in opening out is indicated by a circle in black. Extent opened out primarily is shown with arrows in solid line. Region remaining unaffected by the primary interference, within which the second initiation of opening out is potentially possible, is represented by the region shown by broken line. Arrows indicate the direction along which the secondary opening out proceeds. In every set of diagrams four cases are shown, where the extent of the primary opening out is variable depending upon the position of the initiating point. **b** and **c** represent that chance of remaining of the region unaffected by the primary interference is increased with the arm length and that in the longer arm much time is needed, in compare with the case in the short one, to be opened out completely by the secondary interference of a single primary opening out.

guishable. On one hand, *spatially,* the chance of occurring of two independent primary openings out is increased proportionally to the parameter $(L-k)$, since the values of $(L-k)$ indicate relatively the chance of remaining of a region unaffected by the primary interference of the first initiating point. On the other hand, *in timing relationship* between the openings out, the chance of occurring of two independent openings out is increased inversely to the time elapsed between the first and the potential second initiation of opening out, since the secondary interference of the first primary opening out proceeds progressively with the elapse of time after the critical time at which the first initiation has occurred. Therefore the relative chance of occuring of the second initiation of opening out against the secondary interference of the first primary opening out is likewise proportional to the parameter $(L-k)$. The parameter $(L-k)$ indicates, in this case, relatively the time in which a given arm length is capable to have the second initiating point (Fig. 10b, c). The former factor is confined to the length itself inherent to the individual arms, and the latter to the timing relationship between two initiations of opening out potentially possible within an arm pair. So that they may be called, respectively, length and time factor involved in chiasma formation. These two factors are entirely independent from each other. Consequently, the chiasma frequency within the range $k<L<(k+l)$ must be proportional to

$$(L-k)^2.$$

It is known already that chiasma frequency is increased by speeding up the meiosis by the influence of high temperature. But no change was noticed in the maximum number of interstitial chiasmata formed in a given arm (MATSUURA and HAGA 1942). Therefore the increase in chiasma frequency is necessarily brought about by the decrease in the effect of secondary interference, that is, by the approaching, in time, of the potential second opening out to the first one. The same idea was expressed in the joint work (l. c.) synonymously with the terms "imperfectness or prematurity in the meiotic condition of chromosomes", (p. 410). Accordingly, the degree of the secondary interference can be expressed by the velocity with which the nuclei convert from pachytene to diplotene condition. This diplotene velocity alone seems to be responsible for the variation in the mean chiasma frequencies in a given organism. This factor may be termed velocity coefficient in chiasma formation, for it represents the main coefficient determining the real chiasma frequency in individual cases. Further we must take in consideration that each independent opening out

occurs either equationally or reductionally by chance with the ratio 2E:1R, two different equational and one reductional mode of opening out being possible with equal chance. Thus the probability of forming a chiasma by a meeting of two independent openings out is 2/3. This also participates in determining the real chiasma frequency, though its effect is subsidiary to the velocity coefficient. This second coefficient may be termed as plane coefficient since it determines the realization of a chiasma by the difference or sameness in the plane or mode of opening out between the independent openings out. Compositing these two coeffcients we may have a single coefficient which determines the real chiasma frequencies. The value of this coefficient is easily computable as is shown below. This composite coefficient will be referred to as the frequency coefficient, being represented with f.

Then, the chiasma frequency of any arm pair within the range $k<L<(k+l)$ will be expressed as

$$X_1=f(L-k)^2, \qquad \text{(I)}$$

where X_1 indicates the chiasma frequency of any arm pair of the one-chiasma-length class. From the above equation we find that the chiasma frequency at the length $(k+l)$ is fl^2. Let $c=fl^2$, then we can obtain the value of f simply from the fact that

$$f=\frac{c}{l^2}, \qquad \text{(II)}$$

In the absence of the secondary interference or time factor the number of independent openings out in the critical length $L=(k+l)$ is invariably two, excepting only when an opening out is initiated at the critical point distant with k from the paired kinetochores (Fig. 11a). Therefore the probability of forming a chiasma in this length is very closely 2/3, that is, almost nearly 66.7 chiasmata per 100 arm pairs in the absence of the secondary interference. This frequency represents the maximum which is capable of reaching by the maximum length, $L=(k+l)$, of the one-chiasma-length class, the maximum frequency decreasing with the reduction in arm length. The relation just mentioned cannot be altered by the reverse in time succession of the two openings out. Namely, it is no longer connected with whether the first opening out is initiated at proximal or distal region, that is, 1→2 or 1←2, 1 and 2 representing the regions from left to right respectively in Figure 11a, and the arrows succession in time. The above relationship that two independent openings out occur potentially invariably in the length $(k+l)$ gives a clue to formulate the chromosome length-

chiasma frequency equation in the two-chiasma-length class.

In any length over the limit that $L=(k+l)$ three independent openings out are potentially possible to occur with the exception where the first opening out is initiated at the critical position distant from the paired kinetochores either with k or $(k+l)$.

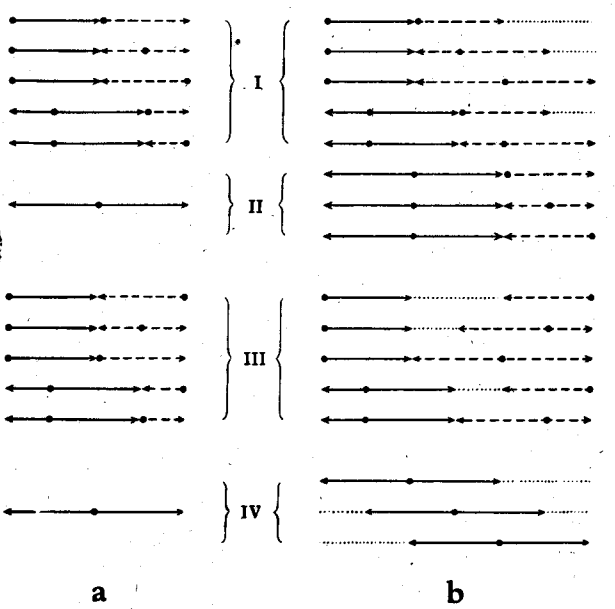


Fig. 11. Comparison of the system of opening out between one- and two-chiasma-length class, maximum lengths $(k+l)$ and $(k+2l)$ being in comparison. Opening out in the former class is shown in **a** and that in the latter in **b**. Initiating point in opening out is indicated by a circle in black. Extent of the primary interference of the first primary opening out is shown with the arrows in solid line, that of the second one with arrows in broken line and that of the third one with dotted line. All the diagrams represent the relation in which the effect of secondary interference is ignored. Therefore the second and third opening out in the diagrams indicate merely the potentialities, their real occurrence depending upon the grade of the secondary interference of the preceding openings out. Relations under all possible combinations of the positions of openings out are shown, three different positions being combined with one another. Comparable cases in those two classes are faced to each other in I, II, III and IV. Time succession in opening out in I and II is 1→2→3, that in III 1→2←3 and that in IV 1←2→3, 1, 2 and 3 representing respectively the regions from left to right in the diagrams, and the arrows succession in time. Of course classification is rather arbitrary. **a**III and **a**IV are simple repetition of the preceding categories.

The possibility of occurring of three independent openings out in this class is proportionally increased with the increase in the parameter $(L-k-l)$. And this relation is not altered by the different time successions in a series of openings out, that is, 1→2→3, 1←2←3, 1→2←3 or 1←2→3, 1, 2 and 3 representing the regions from left to right in Figure 11b, and the arrows succession in time. How short the length may be, chiasma frequencies invariably surpass the maximum frequency in one-chiasma-length class, c, in the present length class. Increase over this least frequency is necessarily brought about by the surplus length $(L-k-l)$. The influence of the interferences in opening out upon the surplus length $(L-k-l)$ is twice the strength as compared with that upon the functional length $(L-k)$ in the one-chiasma-length class. For the length $(L-k-l)$ is influenced by the primary and secondary interferences from the first and second openings out regardless of its position, which may be considered conventionally as proximal, distal, intercalary or at both ends of the arm divided into two portions (Fig. 11b). However, the influence of the interferences in this fashion must be considered to be reduced to the same degree as that in the one-chiasma-length class at the maximum length, where $L=(k+2l)$, of this length class, since at this maximum length the effect of twice strengthened interferences reacts with twice increased functional length $2l$. So that the frequency coefficient for the individual surplus length $(L-k-l)$ must be modified as

$$f\frac{(L-k-l)}{l}.$$

The value of this modified frequency coefficient is increased with the increase in surplus length, attaining to f at the maximum length $(k+2l)$. Chiasma frequency for the constituent length $(k+l)$ is fl^2, that is c, and that for the remaining length $(L-k-l)$ is

$$\frac{f(L-k-l)}{l}(L-k-l)^2,$$

Derivation and meaning of the term $(L-k-l)^2$ for the chiasma frequency of the surplus length $(L-k-l)$ are the same as those in the term $(L-k)^2$ involved in the chiasma frequency of the one-chiasma-length class. Accordingly chiasma frequencies in two-chiasma-length class will be expressed by summing as follows:

$$X_2=c+\frac{f}{l}(L-k-l)^3 \qquad \text{(III)}$$

or $$X_2=f\left[l^2+\frac{1}{l}(L-k-l)^3\right], \qquad \text{(IV)}$$

where X_2 stands for chiasma frequency.

Now suppose two cases with different mean chiasma frequencies, chiasma frequencies of every arm length being expressed with X and X' respectively and the frequency coefficients with f and f' respectively. Then we find from the equations (I) and (IV) the fact that

$$\frac{X'}{X}=\frac{f'}{f}. \qquad \text{(V)}$$

This equation is important in showing that the ratio between the chiasma frequencies in a set of arm pairs is constant regardless of the absolute chiasma frequencies which are variable depending upon the environmental conditions.

Returning to the observed data, we are to test the validity of the above equations. Firstly the method of least squares was applied to the observed chromosome length-chiasma frequency data to obtain the best fitting parabolic line $y=ax^2+bx+c$. There was gained an equation $X=0.734L^2-7.241L+20.370$ for the data in 1937 involving the arms C_s, E_s, B_s and E_1, X and L representing chiasma frequency and arm length respectively. This equation fits fairly good with the observed data. But it does not intersect with the abscissa, therefore failing to estimate the value of k (Table 8). This is obviously due to the absence of arm lengths close to the value of k. On the same reason computable intersection of the lines which fit respectively to the chiasma frequencies of one- and two-chiasma-length class is not reliable to estimate the value of $(k+l)$. Accordingly we must follow another method to estimate the values of k and l. For this

Table 8. A mathematical calculation of a parabolic line which fits best to a part of the observed arm length-chiasma frequency relation (C_s–E_1, 1937).

Length	Freq. cal. (%)	Freq. obs. (%)	Dev.
0.0	20.37	—	—
1.0	13.86	—	—
2.0	8.82	—	—
3.0	5.25	—	—
4.0	3.15	—	—
5.0	2.52	—	—
6.0	3.35	3.0	−0.35
7.2	6.29	6.7	+0.41
10.0	21.36	20.0	−1.36
10.3	23.66	24.8	+1.14

purpose the writer used the following facts: (1) The chiasma frequencies at the critical lengths k, $(k+l)$ and $(k+2l)$ are 0, c and $2c$ respectively as is evident in the equations (I) and (III). So these three values necessarily fall upon a straight line. (2) Chiasma frequencies at the length E_1 and D_1 are sharply rising up and the distances between D_s and E_1 and between E_1 and D_1 are nearly equal, suggesting the closeness of the arm E_1 and D_1 to the length $(k+l)$ and $(k+2l)$ respectively. (3) Therefore, roughly saying, the value of k is expected between the lengths D_s and C_s, and that of $2l$ is somewhat larger than the distance between C_s and D_1, and lastly the length $(k+l)$ must be between E_1 and B_1, but much closer to the length E_1 (cf. Fig. 12a). Basing on these three characteristics, a rule was applied to gain a straight line passing through supposed 0, c and $2c$ points. The distance of 0 point from the origin of co-ordinates representing the value of k and that between 0 and $2c$ point the value of $2l$. Thus the distance between 0 point and a certain point as close as possible to D_1 was divided by 2 to obtain the value of l. After several trials the values which fit most goodly with the observed data were chosen as the approximate values of the constants (Table 9). The value of f was computed by the use of equation (II). With these values theoretical chiasma frequencies were calculated.

Table 9. Values of constant and coefficient determining the chiasma frequency.

Const. & coeff.	(1937)	(1938)
k	4.4	4.4
l	6.2	6.2
f	0.78	1.04
c	30.0	40.0

The observed and calculated chiasma frequencies are in good conformity, substantiating the validity of the foregoing formulation of the chromosome length-chiasma frequency equation (Fig. 12a and Table 10). Further the constancy of the ratio between chiasma frequencies in a set of arm pairs, the relation expressed in the equation (V), was also manifested with the observed data (Table 11).

According to the equation (I) and (IV), two sets of logarithmic chiasma frequencies determined by different frequency coefficients must be parallel when they are plotted against the logarithmic arm lengths. However, logarithmic frequencies of the one- and two-chiasma-length class are

to fall upon the different straight lines as is obvious in comparison between the equation (I) and (IV). In fact these are revealed to be the case (Fig. 12b). This is important in proving that the determination of chiasma frequency is followed by different systems in the one- and two-chiasma-length class. Thus the validity of the foregoing theoretical deduction as to the mechanism of chiasma formation was justified by the data observed.

Table 10. Frequency of interstitial chiasmata in individual arm pairs.

(1937) (600 complete nuclei)

Chromos. arm	Relative length (%)	Xta obs.	Freq. obs. (%)	Freq. cal. (%)	Dev.	P.E.
C_s	6.0	18	3.0	2.0	+1.0	±0.385
E_s	7.2	40	6.7	6.1	+0.6	±0.714
B_s	10.0	120	20.0	24.5	−4.5	±1.184
E_1	10.3	149	24.8	27.2	−2.4	±1.225
B_1	14.1	190	31.7	35.4	−3.7	±1.316
A_2^1	16.0	287.5	47.9	49.8	−1.9	±1.376
C_1	16.1	306	51.0	50.9	+0.1	±1.376
D_1	16.7	367	61.2	58.6	+2.6	±1.355
Total	96.4	1477.5	—	—	—	—

(1938) (400 complete nuclei)

Chromos. arm	Relative length (%)	Xta obs.	Freq. obs. (%)	Freq. cal. (%)	Dev.	P.E.
C_s	6.0	16	4.0	2.7	+1.3	±0.547
E_s	7.2	48	12.0	8.2	+3.8	±0.925
B_s	10.0	103	25.8	32.6	−6.8	±1.581
E_1	10.3	155	38.8	36.2	+2.6	±1.621
B_1	14.1	173	43.3	47.2	−3.9	±1.684
A_2^1	16.0	231	57.8	66.4	−8.6	±1.593
C_1	16.1	294	73.5	67.9	+5.6	±1.574
D_1	16.7	323	80.8	78.1	+2.7	±1.395
Total	96.4	1343	—	—	—	—

Before going further, a brief mention will be made as to the position of chiasmata and the distance between chiasmata. In the one-chiasma-length class a chiasma will be formed proximally to the kinetochore if the first opening out is initiated at any position distant from kinetochore beyond the length $(L-k)$. On the contrary, position of a chiasma will be distal when the first initiating point is happening within the proximal region not exceeding the distance k from the kinetochore. Therefore a chiasma is formed at any possible position throughout the length of an arm, the position being determined passively by two factors, that is, the position of the

Table 11. Ratio between the frequencies of interstitial chiasmata in individual arm pairs.

(1937) (600 complete nuclei)

Chromos. arm	Relative length (%)	Xta obs.	Ratio obs. (%)	Ratio cal. (%)	Dev.	P.E.
C_s	6.0	18	1.2	0.8	+0.4	±0.156
E_s	7.2	40	2.7	2.4	+0.3	±0.269
B_s	10.0	120	8.1	9.6	−1.5	±0.517
E_1	10.3	149	10.1	10.7	−0.6	±0.543
B_1	14.1	190	12.9	13.9	−1.0	±0.607
A_2^1	16.0	287.5	19.5	19.6	−0.1	±0.697
C_1	16.1	306	20.7	20.1	+0.6	±0.704
D_1	16.7	367	24.8	23.0	+1.8	±0.739
Total	96.4	1477.5	100.0	100.1	−0.1	—

(1938) (400 complete nuclei)

Chromos. arm	Relative length (%)	Xta obs.	Ratio obs. (%)	Ratio cal. (%)	Dev.	P.E.
C_s	6.0	16	1.2	0.8	+0.4	±0.164
E_s	7.2	40	3.6	2.4	+1.2	±0.282
B_s	10.0	103	7.7	9.6	−1.9	±0.543
E_1	10.3	155	11.5	10.7	+0.8	±0.569
B_1	14.1	173	12.9	13.9	−1.0	±0.637
A_2^1	16.0	231	17.2	19.6	−2.4	±0.731
C_1	16.1	294	21.9	20.1	+1.8	±0.738
D_1	16.7	323	24.1	23.0	+1.1	±0.775
Total	96.4	1343	100.1	100.1	0.0	—

initiating point of the first opening out and the time elapse during which secondary interference of the first opening out is in action. Similar relation is also maintained in the two-chiasma-length class. But in this length class it will be noticed that distance between chiasmata is variable depending upon the relationships in time and space between the openings out (cf. Fig. 11). On account of this circumstance, the loop between the kinetochore and a chiasma nearest to it may sometimes become shorter than k (D in Figs. 3 and 16). And the loop length between two chiasmata may sometimes shorter than l (A in Fig. 8). These configurations imply nothing other than that these short loops are formed by the opening out which happened late in time succession in the series of openings out. In this specific case the primary interference is suppressed to attain its inherent length between two chiasmata or between the paired kinetochore and a chiasma because these act mechanically to impede the development of opening out across themselves.

The next question concerns the relation of chiasma frequency to the

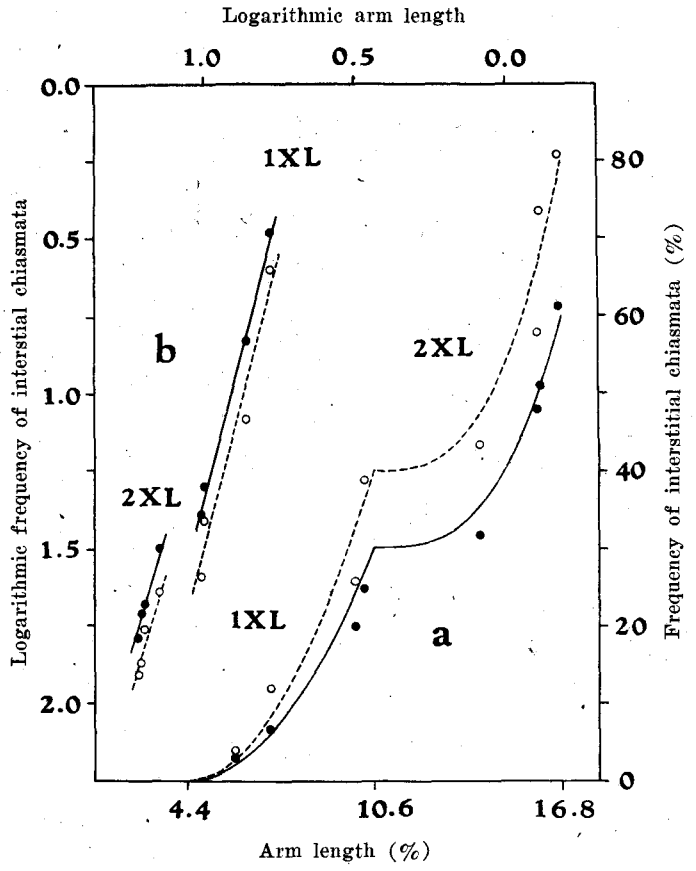


Fig. 12. Relation of frequency of interstitial chiasmata to arm length. **a**, actual chiasma frequencies plotted against the relative arm lengths. **b**, logarithmic representation of the relation in **a**. Circles in black indicate the frequencies observed in 1937 and circles in blank those observed in 1938. Calculated curves in 1937 are shown with solid line, and those in 1938 with broken line. 1XL and 2XL indicate one- and two-chiasma-length class respectively.

chromosome length in which more than two chiasmata can be formed, e.g. three-, four-, five-chiasma-length class and so on. The problem will be dealt with, at first setting aside for the moment the effect of the secondary interference, that is, the time factor. In two-chiasma-length class the maximum number of openings out is two or three depending upon the position of the first initiating point in opening out (Fig. 11b). The same varies between two and four in three-chiasma-length class for the same reason. Generally saying, these maximum numbers are determined obligatorily by

the spatial relationship between the positions at which the primary openings out happened. As pointed out in the simplest pair two- and three-chiasma-length class, there occurs overlapping between the series of maximum numbers of openings out in any pair of adjacent classes. This overlapping becomes increasingly marked with the increase in length (Table 12). Therefore in the present case it is impossible to contrast the two adjacent classes as in the case between one- and two-chiasma-length class. Overlapping in this manner obviously leads to the abolition of the demarcation between adjacent classes, chiasma frequencies of the longer members in a class being continuously and smoothly jointed with those of the shorter members of the next class. For even within a single class shorter members necessarily tend to open out with lower maximum numbers and the longer with higher ones, thus the system of opening out being continuously altered with the length.

Table 12. Series of the maximum numbers of openings out in relation to chromosome length.

Value of $\frac{L}{l}$	Maximum number of openings out										No. of cases
	1	2	3	4	5	6	7	8	9	10	
0—1	+	·	·	·	·	·	·	·	·	·	1
1—2	+	+	·	·	·	·	·	·	·	·	2
2—3	·	+	+	·	·	·	·	·	·	·	2
3—4	·	+	+	+	·	·	·	·	·	·	3
4—5	·	·	+	+	+	·	·	·	·	·	3
5—6	·	·	+	+	+	+	·	·	·	·	4
6—7	·	·	·	+	+	+	+	·	·	·	4
7—8	·	·	·	+	+	+	+	+	·	·	5
8—9	·	·	·	·	+	+	+	+	+	·	5
9—10	·	·	·	·	+	+	+	+	+	+	6
	0	1	2	3	4	5	6	7	8	9	
	Maximum number of Xta to be formed										

Accordingly, the chiasma frequency of the length exceeding approximately the value that $L/l=3$, will be proportional to L^2 regardless of the increase in value of L/l. The meaning of the exponent square is the same as that involved in the chromosome length-chiasma frequency equations for one- and two-chiasma-length class, being derived from length and time factor. Therefore

$$X_{3\text{-}n}=fL^2, \qquad \text{(VI)}$$

where $X_{3\text{-}n}$ indicates chiasma frequency of any length of all ranges from 3- to n-chiasma-length class. Frequency coefficient f in the present case is, of course, not equivalent with that in the foregoing cases of one- and

two-chiasma-length class. Further, the equation (VI) indicates that the ratio between chiasma frequencies is determined simply by the square of inividual length L^2.

Regretfully the above relation was not tested with the data in *Paris verticillata* on account of the absence of the length classes which form more than two chiasmata. However, the relation was confirmed with a specific datum in *Trillium kamtschaticum* (MATSUURA and HAGA 1942). *Trillium kamtschaticum* comprises also only two length classes, one- and two-chiasma-length class, under the normal condition that the kinetochores of a bivalent remain paired until the first anaphase. By high temperature treatment there were induced 128 exceptional bivalents, including several potential bivalents which were in reality in a pair of univalents, in 43 pollen mother-cells (t_4 in the joint work l. c.). In these exceptional bivalents a kinetochore pair of a bivalent appeared separated at first metaphase. Judging from the size of open or closed loop involving the kinetochore, this separation has happened apparently at early prophase. Thus development of the loop in this region is obviously equivalent with the opening out in the other region in relation to the chiasma formation. Hence it is valid to consider that in those exceptional bivalents entire chromosome length has behaved as a single unit in chiasma formation. Length class distribution in those exceptional bivalents is not known. But close similarity of the morphology and behavior of the chromosome complement of *Trillium kamtschaticum* to that of *Paris verticillata* throws light upon this point (cf. HAGA 1934). Namely the class distribution in *Trillium kamtschaticum* will be expressed approximately by that in *Paris verticillata*. Then it seems not far apart from that the exceptional bivalents at issue comprise three-, four and five-chiasma-length class (cf. Table 13).

Table 13. Value of L/l of the individual chromosome of *Paris verticillata*.*

Chromos.	Length		$\frac{L}{l}$
	In micron	In unit (L)	
A	25.3	32.0	5.2
B	19.0	24.1	3.9
C	17.4	22.0	3.5
D	16.1	20.4	3.3
E	13.8	17.5	2.8

* In calculation of L total sum of the entire lengths of chromosome B, C, D, E and a half length of chromosome A, i.e. 79.0 microns, is used as 100 units to keep step with the foregoing calculations.

After these considerations the method of least squares was applied to the chromosome length-chiasma frequency data obtained in the exceptional bivalents in order to find the value of f. The best fitting value of f thus computed was approximately 0.36, the theoretical chiasma frequencies being calculated with the more accurate value 0.3587 to bring close the calculated total number of chiasmata to the observed one. As is natural, good conformity between observed and calculated chiasma frequencies was manifested (Table 14). The observed ratio between chiasma frequencies of the different lengths is also in good conformity with the calculated one (Table 15). The last fact is important in proving the validity of the theoretical consideration, since in this case value of f is utterly excluded from the calculation of the ratio. Accordingly, the theoretical equation (VI) was also substantiated with a good evidence.

Further analyses of chromosome length-chiasma frequency relationship were made with the previous data in *Allium zebdanense* (LEVAN 1935) and in *Crepis capillaris* (RICHARDSON 1935a). In those plants kinetochores of

Table 14. Frequency of interstitial chiasmata in the bivalents of *Trillium kamtschaticum* in which kinetochores were separated before first metaphase (t_4, MATSUURA and HAGA 1942).*

Chromos.	Relative length (%)	II's obs.	Xta obs.	Freq. obs.(%)	Freq. cal. (%)	Dev.	P.E.
A	29.3	17	51	300.0	308.0	− 8.0	±22.375
B	20.1	22	41	186.4	145.0	+41.4	±17.881
C	19.3	23	30	130.4	133.6	− 3.2	±17.320
D	17.3	33	31	93.9	107.4	−13.5	±15.848
E	14.1	33	18	54.5	71.3	−16.8	±13.262
Total	100.1	128	171	765.2	765.3	− 0.1	—

* Including the potential bivalents which were actually in two univalents.

Table 15. Ratio between the frequencies of interstitial chiasmata of the bivalents of *Trillium kamtschaticum* in which kinetochores were separated before first metaphase (t_4, MATSUURA and HAGA 1942).*

Chromos.	Relative length (%)	II's obs.	Xta obs.	Freq. obs.(%)	Ratio obs. (%)	Ratio cal. (%)	Dev.	P.E.
A	29.3	17	51	300.0	39.2	40.2	−1.0	+2.528
B	20.1	22	41	186.4	24.4	18.9	+5.5	±2.019
C	19.3	23	30	130.4	17.0	17.5	−0.5	±1.959
D	17.3	33	31	93.9	12.3	14.0	−1.7	±1.789
E	14.1	33	18	54.5	7.1	9.3	−2.2	±1.498
Total	100.1	128	171	765.2	100.0	99.9	+0.1	—

* See foot-note to Table 14.

the homologous chromosomes are separated probably at early prophase as far as judged from the illustrations. The maximum number of interstitial chiasmata formed in individual chromosomes generally exceeds two, except the bivalent IX in *Allium zebdanense* and C in *Crepis capillaris* in which the observed maximum was one. Accordingly, in these cases entire length of a chromosome must have behaved as a unit in chiasma formation, the length classes being distributed, roughly saying, within the lower portion of the range X_{3-n}. Astonishingly we find in these cases that the ratio between the chiasma frequencies of the different lengths approaches the simple ratio between lengths rather than the ratio between squares of lengths (Tables 16 and 17). Of course, terminal chiasmata in the original data were excluded in the calculation of the present ratios. Discrepancy of the present ratios from the foregoing theoretical consideration is probably due to the fact that in these plants time factor in chiasma formation is nearly negligible, or in other words, that openings out happen nearly simultaneously throughout the length. In this manner of formation chiasma frequency will be proportional rather directly to the chromosome length. In such cases, naturally, chiasma frequencies will be close to the maximum mean frequency which is characteristic to the given organisms. Chiasma frequencies of *Allium zebdanense* and *Crepis capillaris* are markedly higher in compare with those of *Paris verticillata* and *Trillium kamtschaticum*, seemingly supporting the above view.

Table 16. Ratio between the frequencies of interstitial chiasmata of the bivalents of *Allium zebdanense* (20 nuclei, LEVAN 1935).*

Chromos.	Xta obs.	Ratio obs. (%)	Relative length (%)	Ratio cal. (%)	Dev. (1)	Dev. (2)
I	42	17.8	16.7	22.7	+1.1	−4.9
II	36	15.3	14.9	18.0	+0.4	−2.7
III	39	16.5	14.7	17.6	+1.8	−1.1
IV	30	12.7	12.9	13.6	−0.2	−0.9
V	30	12.7	10.8	9.5	+1.9	+3.2
VIII	21	8.9	8.5	5.9	+0.4	+3.0
VI	14	5.9	7.7	4.8	−1.8	+1.1
VII	11	4.9	7.4	4.4	−2.7	+0.3
IX	13	5.5	6.5	3.5	−1.0	+2.0
Total	236	100.0	100.1	100.0	−0.1	0.0

* Terminal chiasmata were excluded intentionally from the original data. Dev. (1) indicates the deviation from the ratio between lengths and Dev. (2) that from the ratio calculated with the formula (VI).

Table 17. Ratio between the frequencies of interstitial chiasmata of the bivalents of *Crepis capillaris* (160 nuclei, RICHARDSON 1935a).*

Chromos.	Xta obs.	Ratio obs. (%)	Relative length (%)	Ratio cal. (%)	Dev. (1)	Dev. (2)
A	260	46.8	43.0	52.5	+3.8	−5.7
D	185	33.3	33.4	31.7	−0.1	+1.6
C	111	20.0	23.6	15.8	−3.6	+4.2
Total	556	100.1	100.0	100.0	+0.1	+0.1

* Average values of the relative lengths calculated from the measurements by MANN (1925), DELAUNAY (1931) and NAVASHIN (1934) were used as the relative lengths. See further foot-note to Table 16.

3. *Origin and behavior of terminal chiasmata*

In the present study prophase was not observed. So there is no available datum to decide directly whether the chiasmata move or not after the time of formation in *Paris verticillata*. According to the comparison between the chiasma frequencies at mid diaphase, late diaphase and metaphase in *Trillium erectum*, a plant cytologically closely similar to *Paris verticillata*, there is little or no indication of movement or terminalization of chiasmata before the first anaphase (HUSKINS and SMITH 1935). The same was further confirmed by an analysis of the chiasma pairs in the same plant (HUSKINS and NEWCOMBE 1941, NEWCOMBE 1941). Secondary chiasmata found in *Trillium kamtschaticum* also reject the possibility of movement of chiasmata in such plants as *Paris* and *Trillium* (MATSUURA 1941b). Basing on these previous observations, it seems highly probable that chiasmata do not move also in *Paris verticillata* before the first anaphase. So that terminal chiasma will be considered, hereinafter, as the terminal junction independent in its origin from the interstitial chiasmata, that is, as the terminal junction in the sense of BELLING (1931, 1933). On the theory of terminalization the terminal chiasmata are assumed to result from terminalization, movement from proximal to distal end, of the interstitial chiasmata (cf. DARLINGTON 1937). However, the phenomenon of terminalization, which is questionable to be of general occurrence, will be disregarded for the moment in this paragraph, a full discussion on this phenomenon being made in the concluding paragraph.

The terminal chiasmata are very infrequent as compared with the interstitial ones in the present plant (Table 5). On account of this circumstance, two data in 1937 and 1938 were summed up to accumulate the data

enough to analyse the relation between arm length and terminal chiasma and that between terminal and interstitial chiasmata (Table 18). This summing up obviously does not affect the conclusion because the following analysis concerns only the relative frequencies.

Table 18. Relation of the frequency of terminal chiasmata to arm length and to number of interstitial chiasmata.

Chromos. arm	Relative length (%)	0X		1X		2Xta		Total TXta	Total arm pairs
		TX (%)	Arm pairs	TX (%)	Arm pairs	TX (%)	Arm pairs		
C_s	6.0	0.4	966	0.0	34	0.0	0	4	1000
E_s	7.2	1.1	912	0.0	88	0.0	0	10	1000
B_s	10.0	4.2	777	0.4	223	0.0	0	34	1000
E_1	10.3	4.6	696	1.0	304	0.0	0	35	1000
B_1	14.1	4.4	641	3.1	355	0.0	4	39	1000
A_2^1	16.0	3.8	510.5	3.3	460.5	0.0	29.0	34.5	1000
C_1	16.1	5.8	446	2.6	508	4.3	46	41	1000
D_1	16.7	7.2	347	4.5	616	2.7	37	54	1000
In total	96.4	3.4	5295.5	2.7	2588.5	2.6	116.0	251.5	8000

Table 19. Simplified representation of the relationship revealed in Table 17.*

Arm length (L)	Number of interstitial chiasmata			Arms of *Paris verticillata*
	0	1	2	
$L<k$	−	−	−	(D_s+D_t)
$L\fallingdotseq k$	+	−	−	—
$k<L<(k+l)$	+	−	−	C_s, E_s
$L\fallingdotseq(k+l)$	+	+	−	B_s, E_1
$(k+l)<L<(k+2l)$	+	+	−	B_1
$L\fallingdotseq(k+2l)$	+	+	+	A_2^1, C_1, D_1

* + indicates the capability of forming a terminal chiasma, and − obligatory incapability of it.

It is evident from the above table that the frequency of terminal chiasmata is increased with the arm length and that the formation of interstitial chiasmata decreases the chance of formation of terminal chiasmata. The relation between terminal and interstitial chiasmata can be summarized as shown in Table 19. Shorter arms within the range $k<L<(k+l)$ never form a terminal chiasma when they have an interstitial one. Near the upper limit of this range, where $L\fallingdotseq(k+l)$, a terminal chiasma is formed,

though very rarely, together with an interstitial chiasma. In the range $(k+l)<L<(k+2l)$ or $L\fallingdotseq(k+2l)$ a terminal chiasma is formed with considerable frequency even when there exists an interstitial chiasma. However in the presence of two interstitial chiasmata it is not formed in the arms of the former range, but in the arms of the latter range though rarely. The meaning of these findings must be sought in that there is required, at least, a certain minimum length of the distal loop to form a terminal chiasma, within which terminal chiasma can never be formed. This minimum is probably expressed by k when there exist no interstitial chiasmata and l when there exist interstitial ones (cf. Table 19). The present minimum length of the distal loop agrees with the distance of the primary interference, k and l, in the diplotene opening out. This fact is very significant in supporting the presumption that the effect of the primary interference is unitary in time and space and not gradual or progressive in its action. For, if the terminal end is passed through by the primary opening out, there remains no chance of forming the terminal junction.

Of course, if the primary opening out commences from the region involving the terminal end, terminal chiasma cannot be formed at all. Presumably the realization of the terminal chiasmata seems to be conditioned, on one hand, by the physical state of the matrix at the time when the interstitial opening out terminated its development at the terminal end of the paired chromosome and, on the other hand, by the grade of repulsion force between separated chromatid pairs which acts against the cohesive effect of the matrix at the terminal junction. If the repulsion force overcomes the cohesive effect of the matrix the terminal junction would be broken off, thus the terminal junction being lost as early as the time of its formation. On the contrary, terminal chiasma would be formed when the repulsion force is failing to break off the terminal junction against the terminal cohesion by virtue of the physical nature of the matrix. Accordingly, balance between these two opposing forces acting at the terminal end seems to be responsible for determining the realization of the terminal chiasma.

The present supposition is supported partly by the writer's own experience on the meiosis of *Spinacia oleracea* (Haga 1935). Majority of the late prophase bivalents of this plant are ring-shaped, both ends being jointed by each one terminal junction. One of these terminal junctions, very probably that of the short arm pair, is seen to be broken at pro-metaphase, thus giving rise to the rod-shaped bivalents at metaphase. This break-

down of a terminal junction is caused apparently by the repulsion force between kinetochores which has been strengthened under the influence of the polar attraction. This process further must have been co-operated by the change in physical state of the matrix, that is, from viscous to non-viscous state.

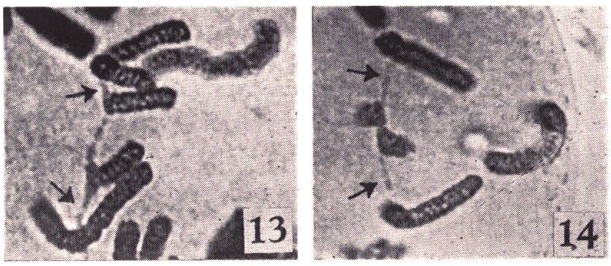
Now arises the question how the frequency of the terminal chiasmata is increased with the arm length. This will be understood with the following explanation. Firstly, increase in arm length decreases the chance of occurring of the primary opening out at the region involving the terminal end, thus the chance of formation of the terminal junction, which may or may not persist, being increased along with the arm length. Secondly, the distal loop closed by a terminal junction will be longer, saying statistically, in the longer arms than in the shorter ones, thus the repulsion force being statistically weak in the longer arms. These two factors, in co-operation with each other, must lead to the higher frequency of the terminal chiasmata in the longer arms. The same interpretation is apparently equally adoptable in the cases where interstitial chiasmata are involved. Remarkable decrease of the frequency of terminal chiasmata with the presence of an interstitial chiasma will be readily explicable as the interstitial chiasma reduces the length of the possible distal loop, thus leading to the increase in power of repulsion acting against the terminal cohesion. This is undoubtedly the reason for the reduced frequency of terminal chiasmata in the case involving an interstitial chiasma. On the same reason presence of the two interstitial chiasmata reduces more severely the frequency of the terminal chiasmata in comparison with the case involving no or one interstitial chiasma.

The fact and interpretation above mentioned is confined to the formation of terminal chiasma in the case where kinetochore is remaining paired until first anaphase, that is, in the case where terminal chiasma participates to form a distal loop closed by itself. The formation of terminal chiasma in the case where kinetochores of the homologous chromosomes are separated at early prophase will follow a somewhat modified way. Apparently there exists no repulsion between paired arms of the open chain configuration, for the repulsion between arms, not between separated kinetochores, is in action always in the lateral direction. So that any terminal junction is realized as terminal chiasma if the cohesive effect of the matrix overcomes the repulsion force between separated kinetochores. Ring-shaped bivalents provided with each one terminal chiasma at both ends will be formed by overcoming of the cohesion of the matrix against the combined effect of

the repulsion force between the kinetochores and between the arms. In certain organisms having small chromosomes the frequency of terminal chiasmata is overwhelming. The terminal chiasmata in those cases seem to be formed, probably, following the development just mentioned above.

Here it is worthy to add some considerations on the physical nature of the matrix. It will be reasonably supposed that matrix changes alternately its rigidity from viscous to non-viscous state and vice versa. Viscous stage is, probably, represented by early pachytene, late diplotene, diakinesis and anaphase. Intermitting stages, late pachtene, early diplotene, and metaphase, are of non-viscous stage. Of course this supposition is a speculation. However, it would be unquestionable that the matrix must be in separable or non-viscous state at the time of opening out or separation of the chromatids. Otherwise they can hardly become free from each other. This inference was supported by certain characteristic figures of chromatid bridges[1].

There were observed break and reunion of chromatids which had taken place very closely to the kinetochore, thus arising the bridges of dikinetic (dicentric) chromatids with almost negligible bridging portion (Figs. 13–14). In those bridges paired daughter kinetochores of a half bivalent are strained to separate under the influence of two opposing forces, that is, polar stress upon the kinetochore of the unaltered monokinetic (monocentric) chromatid and the effect of pulling back of the bridging upon the kinetochore of the dikinetic chromatid (Figs. 13–14). In those cases, as



Figs. 13–14. Bridges at first anaphase showing the viscous nature of the matrix surrounding the paired kinetochores of the half-bivalents. **13**, chromosome B. **14**, chromosome C. In both configurations entire parts of long arms of two chromatids are cast off as non-fused two fragments in **13** and as a fused fragment in **14**. Threads of matrix origin are shown with arrows. ×1460.

1) Full accounts as to the bridge will be given in the next paper of this series.

shown with arrows, a faintly staining fine thread is seen between the forcibly separated kinetochores of a half-bivalent, which are otherwise passing to the pole intimately paired together. Those threads represent nothing other than the matrix, at the viscous state, surrounding the kinetochores. The same state must be undoubtedly retained by the matrix surrounding the kinetochore pairs of the normally separating half-bivalents. This viscous state converts into the non-viscous separable state at the second metaphase as the half-dyads separate freely leaving no connecting threads between separating kinetochores. This fact indicates that the matrix changes its rigidity from viscous to non-viscous state or from inseparable to separable state, keeping step with the change in behavior of kinetochores from non-separating to separating stage. Further evidence for this synchronism or parallelism between the behavior of the matrix and the kinetochore is seen in the characteristic behavior of the precocious bivalent in *Trillium kamtschaticum*. In this specific abnormal bivalent four chromatids undergo the coiling of chromonemata independently from each other, but within the configuration of the bivalents. And all the four chromatids within this bivalent separate freely and pass to the opposite pole with the assortment of chromatids 2–2 or 3–1 at first anaphase (MATSUURA 1937b, MATSUURA and HAGA 1940). Namely, in contrast to the normal bivalent, all the four daughter kinetochores of a precocious bivalent are to separate freely at the first anaphase. In this case no connecting thread is recognized between the separating kinetochores, therefore the synchronization being fulfilled between the behaviors of the matrix and the kinetochore even in this specific case. Moreover it is verified statistically that four daughter kinetochores of the normal bivalents of *Trillium kamtschaticum* assort at random in a pair of paired two kinetochores at first metaphase (MATSUURA 1937a, 1938). In this normal separation of the four daughter kinetochores we find no specific connection as the threads between the forcibly separated kinetochores of the bridge configuration above described. Therefore the state of the matrix surrounding the kinetochores was very probably non-viscous and separable at the late metaphase. And this non-viscous state must have been converted, perhaps suddenly, into the viscous inseparable state at the beginning of the anaphase as we have seen in the bridge configurations.

The above considerations are, critically saying, confined to the behavior of the kinetochore and the matrix surrounding it. But the same relationship, as seen in that chromosome region, should be held unaltered also in the part of the chromonema proper, in so far as the matrix represents the common substance throughout the chromosome without any qualitative

differentiation at kinetochore and the remaining part of the chromosomes. The last assumption will be confident in comparing the behaviors of the daughter kinetochores and the matrix surrounding them in the precocious bivalents with that in the normal bivalents. It is quite certain that the characteristic behavior of the kinetochores and their surrounding matrix in the normal bivalents is brought about by the posteriority in meiotic behavior of the kinetochore in relation to that of chromatid proper, while in the precocious bivalents anomalous first division is brought about by the synchronization in the behavior of the kinetochore and the chromatid proper (MATSUURA 1941a). This comparison is enough to suggest the homogeneity of the martix throughout the chromosome.

As far as the foregoing interpretations are justified, the matrix would change its physical state from non-viscous to viscous state as soon as the diplotene opening out is completed. And the separated matrix embedding separated chromatid pairs does not repulse longitudinally, as is indisputable in the observed facts, the repulsion being in action always laterally. This alteration of the physical nature from non-viscous to viscous state will play a determining rôle in the realization of the terminal chiasmata. If this alteration takes place rapidly, then the chance of maintenance of the terminal junction as a terminal chiasma will be increased because the repulsion force, acting to break off the terminal junction, lessens its influence against the cohesive effect of the viscous matrix. In *Trillium kamtschaticum* it has been disclosed that increase in the frequency of terminal chiasmata per nucleus is closely associated with the increase in the frequency of interstitial chiasmata per nucleus. However, within a bivalent those two frequencies show no association (MATSUURA and HAGA 1942). This close association between the frequencies of the interstitial and terminal chiasmata per nucleus will be readily explained, as is obvious from the foregoings, as follows: High frequency of the interstitial chiasmata is induced by the acceleration of the diplotene velocity, this acceleration will induce indubiously at the same time the rapid alteration of the matrix from non-viscous to viscous state. By this rapid conversion of the physical nature of the matrix much terminal junctions will be realized as the terminal chiasmata in compare to the case of slower conversion. Though some points may be speculative, yet the explanation here mentioned seems to be the most adequate one for the interesting relation above quoted.

All the facts mentioned in this paragraph emphatically point to elucidate the origin of the terminal chiasma with the viewpoint that terminal chiasma has no relation in its origin to the interstitial ones, that

is, to the so-called phenomenon of terminalization. Finally, in this connection, it is noteworthy that ERNST (1940) had figured, in meiosis of haploid *Antirrhinum majus,* the terminal connection between the half-univalents, which is no more distinguishable from the terminal chiasma between the half-bivalents in the diploids. Similar terminal connection was also observed in *Taraxacum* species between univalents which were associated secondarily (CUSTAFSSON 1936). These specific connection must have originated obviously from the matrix, supporting the foregoing interpretation of the origin of the terminal chiasma. Furthermore, development of the "end association" of the bivalents in the female of *Bombyx mori* is also in support of the present conclusion (cf. MAEDA 1939).

4. Frequency of univalent formation in relation to chromosome length

A pair of univalents of the homologous chromosomes was occasionally observed instead of a bivalent at first metaphase, the number of univalent pairs varying from cell to cell. In the great majority of cases the number of pairs was one, more than two pairs appearing very infrequently (Figs. 15–16 and 18). The frequency of pollen mother-cells including such univalent pairs was 2.3 per cent of a total of 5700 pollen mother-cells observed in the year 1937. Accordingly average frequency per chromosome pair is calculated as approximately 0.5 per cent. In the year 1939 the absolute frequency was not estimated, however as far as the writer's experience goes, it does not differ greatly from the frequency obtained in the preceding year.

In certain cases it is known that the univalent formation, partial or nearly complete non-pairing at first metaphase, is caused by the influence of a recessive gene (BEADLE 1930, 1933, BERGNER, CARTLEDGE and BLAKESLEE 1934, KOLLER 1938b, CATCHESIDE 1939, etc.). However, the presence and its influence of such a gene can be hardly supposed in the present case as is convincing in the very low frequency of univalent formation. On the other hand it is noticed in innumerable observations that univalents appear accidentally and exceptionally not only in polyploids but also in diploid of the non-hybrid origin (cf. DARLINGTON 1937, p. 402). The present case of *Paris verticillata* obviously belongs to this latter category. Univalent formation in such cases is generally interpreted as being brought about by the accidental failure in the formation of chiasma, including the terminal chiasma, accompanied by the prophase separation, asynapsis or desynapsis, of the kinetochores of the homologous chromosomes.

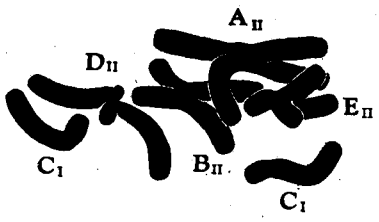


Fig. 15.

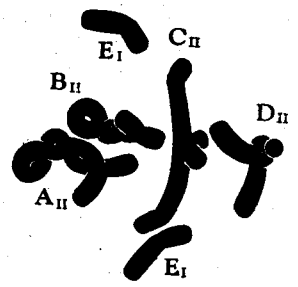


Fig. 16.

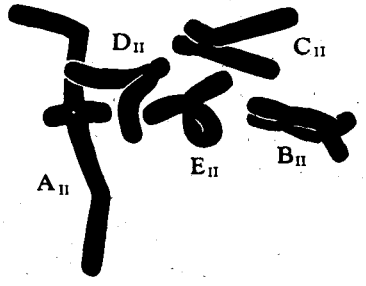


Fig. 17.

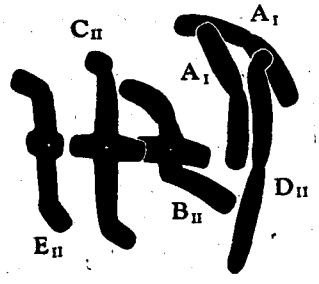


Fig. 18.

Figs. 15–18. Side view of first metaphase. Pair of univalents is seen in **15** (chromosome C), **16** (chromosome E) and **18** (chromosome A). Note the bivalents maintained only by the chiasmata in **17** (chromosome A) and **18** (chromosome B, C, D and E). Configuration of the heteromorphic short arm pair of bivalent DD⁻ is equational in **16** and **17**, and reductional in **15** and **18**. ×1050.

Frequencies of univalent formation of the individual chromosomes are presented in Table 20, in which a pair of univalents of the homologous chromosomes was treated as one because a pair of two univalents represents a single resultant from an univalent formation. It is decisive with the present data that the frequency of univalent formation is inversely proportional to the entire length of chromosome. This is a natural consequence from the fact that frequency of interstitial chiasmata is expressed as a function of the entire length of chromosome when the kinetochores of the homologous chromosomes are separated at early prophase (cf. p. 40). The subsidiary rôle of terminal chiasmata in maintenance of homologous chromosomes in a bivalent is negligible in the present case as the frequency of terminal chiasmata is practically negligible in compare with that of interstitial ones. Therefore the frequency of univalent formation will

be expressed with an inverse function of the frequency of interstitial chiasmata which is in turn a function of the square of chromosome length. Accordingly, relativity of the frequency of univalent formation of a given chromosome in a given set of chromosomes will be expressed by

$$\Sigma L^2 - L^2,$$

where ΣL^2 represents the total sum of the squares of lengths of all the chromosomes composing the complement, and L^2 square of length of a chromosome in question. This term indicates that the shorter the length, relatively the more frequent is univalent formation. On the other hand the frequency of each chromosome is inversely functional to the square of its own length. Consequently relative ratio between the frequencies of univalent formation of the chromosomes of different lengths will be determined approximately with the values computed from

$$U_r = \frac{\Sigma L^2 - L^2}{L^2}, \qquad \text{(VII)}$$

where U_r denotes the relative frequency of univalent formation. The ratio observed in *Paris verticillata* closely approximates to that calculated with the above formula (Table 20). The same ratio in *Trillium kamtschaticum* is found also in good conformity with the calculated ratio (Table 21, cf. MATSUURA and HAGA 1942). However, here it must be especially emphasized that the adoptation of the present formula is confined only to the case where the frequency of terminal chiasmata is negligible against that of the interstitial ones. When the terminal chiasma plays, in its frequency, an important rôle in maintenance of the homologous chromosomes in a bivalent, the above formula is to be written as

$$U_r = \frac{\Sigma X_t - X_t}{X_t}, \qquad \text{(VIII)}$$

where ΣX_t indicates the mean chiasma frequency per nucleus including terminal chiasmata and X_t the mean chiasma frequency including terminal chiasmata of a given chromosome. The test of this formula was made with the data on X-strain, an asynaptic strain, of *Crepis capillaris* (RICHARDSON 1935b). In the normal strain of this plant the mean number of terminal chiasmata per bivalent attains to 0.5 (RICHARDSON 1935a). The same number in the X-strain was not given, but it seems evident that it plays as great a rôle as in normal strain, as far as can be judged from the illustrations. Therefore the data fit for testification of the second formula

for the determination of the relative frequency of the univalent formation. Fairly good approximation of the calculated ratio to the observed one was ascertained (Table 22). So the formulation of the second formula is also substantiated with an actual observation. It is interesting to note that the high frequency of univalent formation in this X-strain of *Crepis capillaris* was assumed to be due to the genic influence, a dominant gene being postulated, in contrast to the purely environmental cause in the cases of *Paris verticillata* and *Trillium kamtschaticum*. Neverthless, the frequency of univalent formation is found to express an inverse function of the chiasma frequency. Accordingly, the present fact is significant in suggesting that the fundamental mechanism of univalent formation is unitary in those different cases, one of which being controlled by genetical factor and the other by environmental one.

Table 20. Ratio between the frequencies of univalent formation of the chromosome pairs of *Paris verticillata*.*

Chromos. pair	Relative length (%)	*U* obs. (1937)	(1939)	Total	Ratio obs.(%)	Ratio cal(%)	Dev.	P.E.
A	27.6	38	30	68	7.4	7.2	+0.2	±0.576
B	20.7	96	52	148	16.1	16.0	+0.1	±0.864
C	19.0	126	70	196	21.4	19.7	+1.7	±0.886
D	17.6	130	77	207	22.6	23.6	−1.0	±0.946
E	15.1	202	96	298	32.5	33.6	−1.1	±1.052
Total	100.0	592	325	917	100.0	100.1	−0.1	—

* *U* indicates pair of univalents.

Table 21. Ratio between the frequencies of univalent formation of the chromosome pairs of *Trillium kamtschaticum* (t_0, MATSUURA and HAGA 1942).*

Chromos. pair	Relative length (%)	*U* obs.	Ratio obs. (%)	Ratio cal. (%)	Dev.	P.E.
A	29.3	13	5.7	5.6	+0.1	±1.030
B	20.1	18	7.9	16.3	−8.4	±1.655
C	19.3	50	22.0	17.9	+4.1	±1.718
D	17.3	65	28.6	23.3	+5.3	±1.895
E	14.1	81	35.7	37.0	−1.3	±2.164
Total	100.1	227	99.9	100.1	−0.2	—

* See foot-note to Table 20.

Table 22. Ratio between the frequencies of univalent formation of the chromosome pairs of X-strain of *Crepis capillaris* (RICHARDSON 1935a, b).*

Chromos. pair	Relative length (%)	*U* obs.	Ratio obs. (%)	Ratio cal. (%)	Dev.	P.E.
A	43.0	85	23.1	18.8	+4.3	±1.374
D	33.4	127	34.5	33.0	+1.5	±1.653
C	23.6	156	42.4	48.2	−5.8	±1.757
Total	100.0	368	100.0	100.0	0.0	—

* See foot-notes to Tables 17 and 20.

Now it will be worth discussing the question as to the developmental process of the failure of chiasma formation which leads to the univalent formation. In this respect two principal modes are distinguishable, of which one is the complete asynapsis at the stage of pairing and the other the desynapsis of the homologous chromosomes which have once really synapsed entirely or, at least, partially along their length (cf. MATSUURA 1937b, MATSUURA and HAGA 1940, etc.). The former fashion of univalent formation is not infrequent under the influence of environmental effects such as low or high temperature. As a matter of fact univalent formation in the case of complete asynapsis has no connection whatever with the chromosome length, because the complete asynapsis in the nucleus is caused through the alteration of the system of division from meiotic to mitotic. This is a fact manifested in an asynaptic type of division in pollen mother-cells of *Trillium kamtschaticum* (MATSUURA l. c., MATSUURA and HAGA l. c.). Accordingly, univalent formation in such manner belongs out of the present scope of discussion. On the contrary, in the desynaptic univalent formation its frequency is to be associated with the chiasma frequency, that is, with the chromosome length. But, if there is a large length difference in the complement, there remains a possibility of the univalent formation in the short chromosome to be sometimes completely asynaptic, complete asynapsis having happened partially in the nucleus. Yet such case of asynapsis should be regarded potentially as desynapsis, since the system of division remains still meiotic.

The behavior of the heteromorphic chromosome pair DD^- of *Paris verticillata* is very instructive in elucidating the finer points in the desynaptic univalent formation. One of the univalents of this particular pair was provided invariably with a head, satellite itself, and the other showed no sign of this appendage, unquestionably the former representing satellited chromosome D and the latter non-satellited D^- (Fig. 19). Further we have

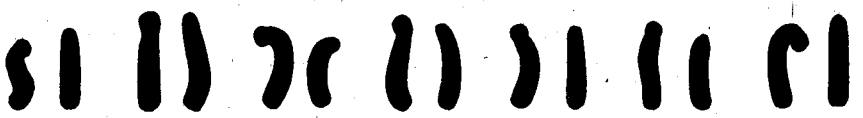


Fig. 19. Seven pairs of first metaphase univalents of the heteromorphic chromosome pair DD⁻. Satellited chromosome D is shown on the left and non-satellited D⁻ on the right in each pair. ×1050.

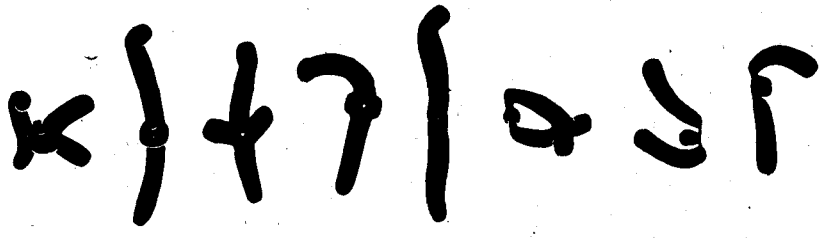


Fig. 20. Eight examples of the exceptional bivalent of the heteromorphic chromosome pair DD⁻. In five bivalents on the left separation of the kinetochores is reductional, satellited chromosome D being shown upwards in each case. In three bivalents on the right, separation of the kinetochores is equational, thus the disjoining of the partners being arrested even when there exists no chiasma. ×1050.

already seen that the relative frequency of univalent formation of this particular pair is involved simply in a series determined by the formula (VII) (cf. Table 20). These two facts authenticate the inference that the kinetochore and its neighboring region were asynaptic even at the end of pairing stage. Therefore the univalent formation is accomplished when this specific localized asynapsis is accompanied by the complete desynapsis, reductional opening out of the really or potentially synapsed distal regions forming no chiasma, interstitial as well as terminal. Opening out in this case may be completed by the single effect of repulsion of the asynaptic loop or by the reductional primary opening out happened in the synapsed distal regions. Most likely the former is responsible for the univalent formation in the majority of cases. When the interstitial or terminal or both of the chiasmata are formed in this specific case of opening out, exceptional bivalents are resulted, in which kinetochores are separated reductionally but the homologous chromosomes are maintained in bivalent by the interstitial or terminal or both of the chiasmata (Fig. 20).

Otherwise, no clue can be found to explain the above observations. If the kinetochore and its neighboring region are effectively synapsed, there must arise equational opening out of the heteromorphic short arm pair twice as frequent as the reductional opening out (cf. p. 20). This equatio-

nal opening out can not result in univalent formation as the lack of satellite in chromosome D⁻ mechanically arrests the disjoining of the partners, the two chromosomes being jointed together by the inseparable paired chromatids of the satellite of chromosome D (Fig. 20). Whereas in the homomorphic pairs there arises no such differential consequence whether the opening out is equational or reductional by virtue of their structural identity. Then, if this is the case, the frequency of univalent formation in the particular chromosome pair DD⁻ is to be much less than the observed one, roughly saying, to be one third of the observed one. For the frequency in chromosome pair DD⁻ is found in concurrence with those in the remaining homomorphic pairs. Therefore the alternative assumption, desynapsis of the kinetochore pair, conflicts with the fact established. Reversely speaking, the frequencies of univalent formation in the homomorphic chromosome pair are in concurrence with that of the heteromorphic chromosome pair DD⁻. This implies nothing other but that the kinetochore and its neighboring regions were also asynaptic in the case of homomorphic pairs. Accordingly it is valid that the univalent formation in *Paris verticillata* is raised generally by the asynapsis of the kinetochores and the regions neighboring them accompanied by the complete desynapsis of the distal regions. It seems highly probable to suppose the same mechanism of univalent formation likewise in the other cases, where the frequency of univalent formation is inversely associated with the chromosome length, e.g. *Trillium kamtschaticum* and *Crepis capillaris*. The extent of asynapsis will be of variable size. In *Paris verticillata* it exceeds generally, at both sides of the kinetochore, the region within the distance of the short arm length of chromosome D, as is evident in the above findings. But, though very rarely, synapsis seems to occur within that region as is shown in the bivalent configuration in which partners are maintained merely by the mechanical property of the unmated satellite (Fig. 20). This characteristic configuration was noticed only several times in the whole series of the present observations. Critically saying, it is certain that this characteristic configuration originates in desynapsis of the kinetochores.

Lastly it must be pointed out that the assumption that all the univalents are raised by the asynapsis of the entire chromosome length fails to account for the causal relationship between the univalent pair and the bivalent maintained only by the chiasmata, and to explain the length-frequency relationship in univalent formation. Furthermore it contradicts the chiasma frequency-chromosome length relationship revealed in the present study. At any rate the present interpretation of the univalent formation can not

be affected even though certain proportions of the univalents are raised by the complete asynapsis of the homologous chromosomes, because such case of asynapsis is to be regarded as potential desynapsis, in so far the division is meiotic.

5. *Intra-nuclear correlation between bivalents in chiasma formation*

Genetically it is known in *Drosophila* species that the prevention of crossing over in a chromosome pair by the presence of heterozygous inversion in it results in increased crossing over in another normal one (MORGAN, BRIDGES and SCHULTZ 1932, 1933, STEINBERG 1936, 1937, MACKNIGHT 1937). Further in *Drosophila* species the existence of such negative correlation between the frequencies of crossing over was established even between the chromosome pairs which are heterozygous each for an inversion (GLASS 1933, SIDOROW, SOKOLOW und TROFIMOW 1936). On the other hand, no such correlation was ascertained in the normal chromosomes of *Primula sinensis* and *Pisum sativum* (DE WINTON and HALDANE 1935). Hence it is not certain as yet whether the *Drosophila* data quoted above represent the phenomenon which occurs universally under the prevention of crossing over in a chromosome pair or a specific one resulted under the influence of inversions which were used to prevent crossing over.

In spite of this uncertainty in the genetical field, it will be interesting to examine cytologically whether there exists or not the similar phenomenon in chiasma formation. Indeed this was done by several workers to demonstrate a parallel between chiasma formation and crossing over (DARLINGTON 1933, SAX 1935, MATHER 1936a, 1939, MATHER and LAMM 1935, LAMM 1936, BENNETT 1938). But the results were very divergent as is seen in the data compiled by MATHER (1936a). The conclusion of the last worker is as follows: "Negatively correlated chiasma frequencies occur more often, but are not universal". "Some species showed no correlation, some had individuals with and others without correlations, and some even had correlations between some bivalents and not between others of the same nuclei" (p. 226). On the basis of the data in *Secale cereale* he has postulated to interpret the negative correlation "that there is an upper limit to the number of chiasmata which can form, in any nucleus, the limit varying slightly from nucleus to nucleus. The bivalents or other configurations must compete for these chiasmata. What determines the limit is not known,...." (p. 222). Further, according to him, positively correlated chiasma frequencies are attributable to "mixed material, or

Table 23. A survey of intra-nuclear correlation

Species	$\bar{n}$	No. of nuclei	Comparison x v. y
Eremurus spp. (2)	7	19	5 long v. 2 short
" " (3)	7	36	" "
" " (1)	7	40	" "
Crocus biflorus	4	101	2 long v. 2 short
Crocus Kowolkowii	10	25	2 long v. 8 short
Culex pipiens (2)	3	42	2 long v. 1 short
" " (4)	3	17	" "
" " (5)	3	18	" "
" " (6)	3	19	" "
Fritillaria chitralensis	12	50	long v. short arm (SM_{II})
Locusta migratoria	11	70	2 long v. 6 medium
Secale cereale	8	36	7 long v. 1 short
Vicia faba (1)	6	60	1 long v. 5 short
" " (2)	6	50	" "

* All the data are inclusive of terminal chiasmata. The data in *Fritillaria* statistics by BENNETT (1938), DARLINGTON (1933) and SAX (1935). All the others r correlation coefficient and $P.E._r$ probable error of correlation coefficient. The

environmental effect". And the intricate cases showing diversity even within the same nucleus were explained by a speculative specialization of the above postulation of "competition".

Previous observations in which inter-class analysis of the correlation is applicable are compiled in Table 23. The value of correlation coefficient (r) and its probable error ($P.E._r$) was calculated in each case. It will be noticed in these data that the calculated correlation coefficients may be negative or positive, but they are all not significant as far as judged with the value of their probable error. Accordingly, no definite conclusion can be drawn from these data as to the question of intra-nuclear correlation in chiasma formation. Further it is important to add here that in those analyses terminal chiasmata were not distinguished from the interstitial ones.

Further analyses were made with the writer's own data in *Paris verticillata* and in *Trillium kamtschaticum* (Tables 24 and 25). The data in the latter plant are a part of those which were obtained but not published in the joint work by MATSUURA and HAGA (1942). Those two plants are

between bivalents in chiasma formation.*

M±σ x		M±σ y		V x	V y	Mean Xta per nucleus	r	P.E.$_r$
2.80	±2.23	1.58	±0.59	80	37	17.16	+0.339	±0.137
2.80	±1.45	1.64	±0.56	52	34	17.28	+0.206	±0.108
2.87	±2.19	1.61	±0.69	76	43	17.57	+0.099	±0.106
2.52	±0.90	1.61	±0.89	36	55	8.26	+0.170	±0.065
2.98	±1.29	1.55	±1.02	43	66	18.36	+0.404	±0.113
1.19	±0.53	1.02	±0.15	45	15	3.40	+0.180	±0.103
1.50	±0.59	1.31	±0.41	39	31	4.31	+0.244	±0.154
1.68	±0.63	1.29	±0.40	38	31	4.65	−0.094	±0.158
1.79	±0.53	1.34	±0.41	30	31	4.92	+0.513	±0.114
3.70	±1.24	1.78	±0.67	34	38	5.48	−0.152	±0.093
2.15	±0.59	1.13	±0.83	27	73	11.08	+0.273	±0.075
2.42	±0.29	0.83	±0.77	12	93	17.77	−0.330	±0.100
6.75	±1.40	2.63	±0.34	21	13	19.90	−0.05	±0.09
7.06	±1.12	3.42	±0.26	16	8	24.16	−0.079	±0.095

chitralensis, Secale cereale and *Vicia faba* (1) were based respectively on the are those compiled by MATHER (1936a). M indicates mean, V variation coefficient, same denotations were also adopted in the following tables.

closely similar, even in the detailed points, in the behavior in chiasma formation (cf. MATSUURA and HAGA l. c.). Thus the two sets of data were combined in the following considerations.

In the present analysis frequency of interstitial chiasmata, excluding terminal ones, of bivalent A was compared with the combined frequency of interstitial chiasmata of the remaining four bivalents. Here we find again that the correlation can be negative or positive, but they are all not significant as the value of probable error indicates in each separate case (Tables 24–26). However, in the present series of data it was disclosed the interesting fact that decrease in variation coefficients (V) in the number of chiasmata formed is accompanied by the increase of the mean number of chiasmata per nucleus. In other words, increase in the mean number of chiasmata per nucleus acts to stabilize the variation in the numbers of chiasmata formed within the nuclei. The same relationship is also recognizable in some of the previous observations, e.g., *Eremurus spectabilis, Vicia faba* and *Culex pipiens,* although there is some limitation in its significance as the terminal chiasmata were involved in the analysis (cf.

Table 24. Number of interstitial chiasmata in bivalent A in relation to the total number of them in the remaining four bivalents of the same nuclei in *Paris verticillata*.

(1937)

Number of Xta in A	Sum of Xta in B to E 0	1	2	3	4	5	6	7	Total nuclei
0	20	53	56	35	12	3	—	—	179
1	30	79	69	68	25	6	2	—	279
2	14	25	49	29	10	3	—	1	131
3	1	2	5	1	—	1	—	—	10
4	—	—	1	—	—	—	—	—	1
Total nuclei	65	159	180	133	47	13	2	1	600

$r = +0.061$. $P.E._r = \pm 0.027$.

(1938)

Number of Xta in A	Sum of Xta in B to E 0	1	2	3	4	5	6	7	Total nuclei
0	3	10	20	24	19	7	1	1	85
1	10	18	47	61	40	14	2	—	192
2	4	18	27	26	23	6	—	—	104
3	1	2	3	3	4	1	—	—	14
4	—	—	—	1	4	—	—	—	5
Total nuclei	18	48	97	115	90	28	3	1	400

$r = -0.028$. $P.E._r = \pm 0.034$.

Table 25. Number of interstitial chiasmata in bivalent A in relation to the total number of them in the remaining four bivalents of the same nuclei in *Trillium kamtschaticum*.

(t_0)

Number of Xta in A	Sum of Xta in B to E 0	1	2	3	Total nuclei
0	192	56	4	1	253
1	29	14	2	—	45
2	2	—	—	—	2
Total nuclei	223	70	6	1	300

$r = +0.069$. $P.E._r = \pm 0.039$.

(t_1) **Table 25.** (*continued*)

Number of Xta in A	Sum of Xta in B to E 0	1	2	3	4	Total nuclei
0	27	7	8	3	2	47
1	4	7	7	—	2	20
2	1	—	2	—	—	3
3	—	—	—	—	1	1
Total nuclei	32	14	17	3	5	71

$r = +0.317$. P.E.$_r = \pm 0.072$.

(t_2)

Number of Xta in A	Sum of Xta in B to E 0	1	2	3	4	5	Total nuclei
0	21	22	25	12	2	—	82
1	6	13	17	13	2	1	52
2	3	2	8	3	1	—	17
Total nuclei	30	37	50	28	5	1	151

$r = +0.199$. P.E.$_r = \pm 0.053$.

(t_3)

Number of Xta in A	Sum of Xta in B to E 0	1	2	3	4	5	6	Total nuclei
0	—	1	5	8	4	2	2	22
1	1	2	13	8	7	7	3	41
2	1	2	3	8	4	2	—	20
3	1	1	1	2	1	2	—	8
Total nuclei	3	6	22	26	16	13	5	91

$r = -0.114$. P.E.$_r = \pm 0.070$.

(t_4)

Number of Xta in A	Sum of Xta in B to E 1	2	3	4	5	6	7	Total nuclei
1	1	1	—	—	—	—	—	2
2	1	2	7	10	1	1	2	24
3	—	—	1	2	5	1	—	9
4	—	—	3	1	2	1	1	8
Total nuclei	2	3	11	13	8	3	3	43

$r = +0.366$. P.E.$_r = \pm 0.089$.

Table 26. Intra-nuclear correlation between bivalents in chiasma

Species	n	No. of nuclei	Comparison x v. y
Paris verticillata (1937)	5	600	A v. B–E
" " (1938)	5	400	" "
Trillium kamtschaticum (t_0)	5	300	" "
" " (t_1)	5	71	" "
" " (t_2)	5	151	" "
" " (t_3)	5	91	" "
" " (t_4)	5	43	" "

Table 23). In addition we see that the sign of correlation coefficients changes from positive to negative, after a certain level of the mean number of chiasmata was reached (Table 26 and Fig. 21). This is quite consistent as far as the present series of data goes. However, on this particular occasion the data in t_4 of *Trillium kamtschaticum* must be excluded, because in this case the system of chiasma formation is entirely different from that in the other cases, in about sixty per cent of the homologous chromosome pairs kinetochores being separated before metaphase.

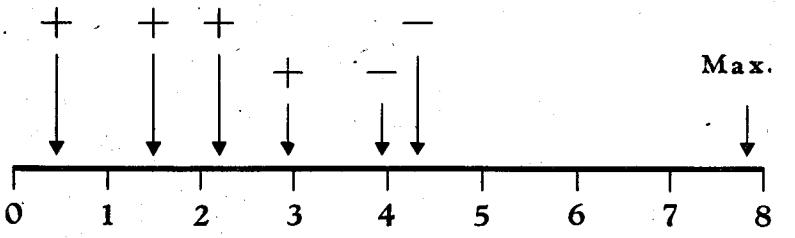


Fig. 21. Sign of correlation coefficient at different levels of the mean number of chiasmata per nucleus. Short arrows indicate the cases in *Paris verticillata* and long ones those in *Trillium kamtschaticum*. Max. near the right end indicates the maximum mean number of chiasmata in *Paris verticillata*.

As pointed out above, the variation in the number of chiasmata formed within the nuclei is inversely correlated with the mean number of chiasmata per nucleus. The stabilizing of the variation is more marked when the mean number of chiasmata is higher. Now then it seems significant that the sign of correlation coefficients changes from positive to negative at a certain level of the mean number of chiasmata per nucleus, that is, between 2.94 and 3.94 in *Paris verticillata*. The positive sign in the case of lower

formation in *Paris verticillata* and *Trillium kamtschaticum*.

M±σ				V		Mean Xta per nucleus	r	P.E.r
x		y		x	y			
0.96	±0.77	1.98	±1.23	80	62	2.94	+0.061	±0.027
1.16	±0.84	2.78	±1.44	72	52	3.94	−0.028	±0.034
0.16	±0.39	0.28	±0.51	244	182	0.45	+0.069	±0.039
0.41	±0.64	1.08	±1.22	156	113	1.49	+0.317	±0.072
0.57	±0.69	1.63	±1.13	121	69	2.20	+0.199	±0.053
1.15	±0.89	3.15	±1.42	77	45	4.31	−0.114	±0.070
2.53	±0.84	4.00	±1.43	33	36	6.53	+0.366	±0.089

mean number probably implies that increased number of chiasmata of a chromosome pair or of a group of pairs is resulted, statistically saying, by the general acceleration of the meiotic velocity in the nuclei. Therefore increased number in one side of the pair in comparison is accompanied by the simultaneous increase in the other side. On this reason positive sign will be gained by calculation, the larger variation in the numbers of chiasmata formed within the nuclei being comprehensible with the less uniformity in the meiotic velocity. While in the case of higher mean number of chiasmata the meiotic velocity is more uniform as is deducible from the decreased variation coefficient in the number of chiasmata formed. Hence in the latter case the increased or decreased number of chiasmata in one side of the pair in comparison probably indicates, in the majority of cases, nothing other than a number deviated from the mean. The other side of the pair in comparison, however, will remain most frequently near the mean number. On this reason negative sign will be calculated from the data. According to these considerations, if any correlation exists, only the positive correlation represents the real relationship, the negative one showing merely the resultant of calculation having no biological significance. In support for this view the value of probable error is much larger in the case of negative correlation than in the case of positive one.

Now arises a question as to what stabilizes the variation in the number of chiasmata formed within the nuclei. As is evident in the actual data, the approach to an upper limit of the number of chiasmata which can be formed within a nucleus lessens the extent of variation. This is apparently due to the diminished size in the range of variation by means of enhancing the lower level. The upper limit is determined inherently by the length or distance of the primary interference in diplotene opening out. The

distance of the primary interference is constant even under different environmental conditions. By virtue of this constancy of the primary interference the maximum of the mean number of chiasmata is necessarily also constant for a given organism. The maximum mean is attained when the effect of time factor, the secondary interference, is nullified in the process of chiasma formation, that is, when there was realized the most rapid completion of the diplotene process. Accordingly, the maximum mean number in *Paris verticillata* will be calculated as follows: We see already that in the absence of time factor the number of openings out is invariably two in the length in which $L=(k+l)$, except only when the opening out is initiated at the critical point dividing the length into two portions of the length k and l respectively. Therefore the probability of forming a chiasma is almost nearly 2/3 in this critical length. For there are three possible ways in opening out, and a chiasma results from the meeting of openings out which are different in their mode of opening out. Then the same probability of the fractional length in which $k<L<(k+l)$, that is one-chiasma-length class, will be

$$\frac{2}{3}\left(\frac{L-k}{l}\right),$$

because the probability of forming a chiasma is 0 in the length k and 2/3 in the length $(k+l)$. In the same manner the probability of forming chiasmata in the two-chiasma-length class should be

$$\frac{2}{3}+\frac{2}{3}\left(\frac{L-k-l}{l}\right)$$

or simply

$$\frac{2}{3}\left(\frac{L-k}{l}\right),$$

where $\frac{2}{3}\left(\frac{L-k-l}{l}\right)$ represents the probability of forming a chiasma by the fractional surplus length $(L-k-l)$.

The probability in each separate arm is calculated with the above formula (Table 27). Summing up all those probabilities, there was obtained the maximum mean number 7.82 per nucleus. The same value for *Trillium kamtschaticum* probably does not differ greatly from the above for *Paris verticillata*. Then, that the change in sign of correlation coefficient takes place between the mean number 2.94 and 3.94, is very signi-

Table 27. Maximum mean number of interstitial chiasmata in *Paris verticillata*.

Chromos. arm	Relative length (%)	Maximum mean
C_s	6.0	0.17
E_s	7.2	0.30
B_s	10.0	0.60
E_l	10.3	0.63
B_l	14.1	1.04
$A_{\frac{1}{2}}$	16.0	1.25
$A_{\frac{1}{2}}$	16.0	1.25
C_l	16.1	1.26
D_l	16.7	1.32
Total	—	7.82

ficant in revealing that the change occurs at an approximately middle level in the whole range of variation in the mean number of chiasmata per nucleus.

Finally, intra-chromosomal correlation between the arms of bivalent A was analyzed in *Paris verticillata*. In this bivalent two arm pairs are indistinguishable from each other by their length. So that the frequency of the dissimilar pairs as to the number of chiasmata, such as 1 and 0 chiasma respectively in each arm pair, was divided by 2 to construct the correlation table (Tables 28–29). There were found no significant correlations (Table 29). The sign of the calculated correlation coefficient was

Table 28. Frequency of different pairs of the numbers of interstitial chiasmata in two arms of bivalent A in *Paris verticillata*.*

No. of Xta in each arm	Frequency (1937)	Frequency (1938)
0 — 0	179	85
1 — 0	279	192
1 — 1	122	91
2 — 0	9	13
2 — 1	10	14
2 — 2	1	5
Total	600	400

* Numbers in two arms of a bivalent are joined with —.

Table 29. Number of interstitial chiasmata in an arm in relation to that in the other arm of the same bivalent A in *Paris verticillata*.

(1937)

No. of Xta in an arm	No. of Xta in the other 0	1	2	Total arms
0	179.0	139.5	4.5	323.0
1	139.5	122.0	5.0	266.5
2	4.5	5.0	1.0	10.5
Total arms	323.0	266.5	10.5	600.0

$r = +0.049$. $P.E._r = \pm 0.027$.
Mean Xta per arm $= 0.48$. $\sigma = \pm 0.53$. $V = 110$.

(1938)

No. of Xta in an arm	No. of Xta in the other 0	1	2	Total arms
0	85.0	96.0	6.5	187.5
1	96.0	91.0	7.0	194.0
2	6.5	7.0	5.0	18.5
Total arms	187.5	194.0	18.5	400.0

$r = +0.031$. $P.E._r = \pm 0.034$.
Mean Xta per arm $= 0.58$. $\sigma = \pm 0.67$. $V = 116$.

positive in contradistinction to the negative sign in the similar case in *Fritillaria chitralensis* (BENNETT 1938, cf. Table 23). However, nothing can be concluded from such unreliable data.

General Conclusion

Meiosis is conditioned primarily by the mode and amount of the pairing of the homologous chromosomes. Hence the present concluding consideration will be entered from the question relating to chromosome pairing. However, non-homologous pairing as manifested in *Zea mays* (MCCLINTOCK 1933), *Nicotiana tabacum* (LAMMERT 1934) and *Antirrhinum majus* (ERNST 1940) will be left out of the consideration, because it results, usually, in no effective pairing. Of course complete asynapsis for the whole nucleus is out of the scope of the present consideration.

In many organisms it is supposed that the pairing is complete along the entire length of chromosomes, that is, the standard mode of chromosome pairing. This condition is inferred, though indirectly, in *Trillium kamtschaticum* by the fact that cross and parallel association take place with the

ratio 2 cross: 1 parallel, which is predicted from the principle of neo-two-plane theory when the chromosome pairing is complete (MATSUURA 1937a, 1938). Direct observation of zygotene and leptotene in *Trillium erectum* by HUSKINS and SMITH (1935) is partly in support for this inference[1]. Thus the pairing in *Paris verticillata,* which is cytologically closely similar to the preceding plants, seems very probable to be complete along the entire length of chromosomes including the kinetochores. In exceptional cases, however, the small region containing the kinetochore is asynaptic throughout the stage of pairing. This is the fact manifested in the cases of univalent formation. Therefore two modes of pairing are confirmed in *Paris verticillata,* that is, (1) complete pairing along the entire length, (2) pairing of all portions except the region containing the kinetochore. The latter type is raised probably by the residual effect of the mitotic nature of kinetochore. In mitosis the division of kinetochores is generally posterior to that of chromonema proper and the kinetochores of different chromosomes repulse each other. The conversion of physiological property from mitotic to meiotic condition is seemingly posterior in kinetochore in comparison to the chromonema proper. Then the residual repulsion between kinetochores of the homologous chromosomes, which resulted from delayed conversion from mitotic to meiotic condition, will lead sometimes to the asynapsis of the region containing the kinetochores, synaptic attraction being overcome with the persisting repulsion force between the kinetochores.

Thus two modes of chromosome pairing are distinguished with regard to the region of kinetochores, in one of which kinetochores of the homologous chromosomes are synaptic, and in the other they are asynaptic. Asynapsis of the kinetochore has happened in *Paris verticillata* partially and exceptionally in the nuclei. This is probably due to the slight upset in synchronization between the kinetochores in transition from mitotic to meiotic condition. Such a partial lack of synchronization in behavior of kinetochores is a fact already observed in mitosis as well as in meiosis (cf. DARLINGTON 1936a, Fig. 17 in DARLINGTON 1937, UPCOTT 1939). Generally

1) These workers remark as follows: "Before synapsis is completed in all regions, the earliest synapsed chromomeres begin visibly to split. There is therefore no definite zygotene stage for the whole nucleus" (p. 121). "Pachytene likewise is not a definite stage for the whole nucleus" (p. 122). However, the same seems not likely to occur in *Paris verticillata* and *Trillium kamtschaticum.* For the mode of chiasma formation is markedly different in the above two plants from *Trillium erectum* (cf. HUSKINS et al. 1935, 1941, NEWCOMBE 1941). Especially in the former plants the chiasma frequency is remarkably low, suggesting the prolonged duration of a stage and gradual transition from one stage to another throughout the entire chromosome length.

Table 30. Proportion of equational separation of the heteromorphic chromosome pairs at first division of meiosis.*

Organism	No. of pairs obs.	E (%)	Investigator
Plant (Autosome):			
Aloe mitriformis	12	25	RESENDE (1936, 1937)
Aloe variegata × *Gasteria verrucosa*	112	37.5	SATÔ (1942)
Paris verticillata	1000	66.7(IM)	HAGA (Present paper)
" "	2000	82.3(IA)	" (")
Secale cereale	402	98 (IA)	KATTERMANN (1939)
Triticum vulgare (a)	94	0	HUSKINS and SPIER (1934)
" " (b)	39	10.3	" and " (")
" " (c)	48	22.9	" and " (")
Zea mays	—	0	MCCLINTOCK (1933)
Animal (Autosome):			
Amphitornis bicolor	—	99	CAROTHERS (1931)
Arphia simplex	(25)	0	" (1913)
Brachystola magna	(300)	0	" (")
Cricetus auratus	253	18.6(MI)	KOLLER (1938a)
" "	111	21.6(AI)	" (")
Mecostethus gracilis (1)	55	50	CAROTHERS (1931)
" " (2)	150	88.9	" (")
" " (3)	63	92.3	" (")
Phrynotettix magnus (c_1)	928	49.2	WENRICH (1916)
" " (b, c_2, c_3)	—	100	" (")
Putorius furo	—	32	KOLLER (1936)
Trimerotropis crinita (1)	—	5	CAROTHERS (1931)
" " (2)	ca. 300	90	" (")
" *suffusa*?	(100)	0	" (1917)
Animal (Sex chromosome X–Y):			
Apodemus agrarius	—	100	MATTHEY (1938)
" *hebridensis*	—	0	KOLLER (1941)
" *sylvaticus*	—	92	" (")
" "	—	80	MATTHEY (1938)
Arvicola sherman	—	50	" (")
Mus musculus	182	0(AI)	MAKINO (1941)
Rana temporaria	—	100	WITSCHI (1924)
Rattus norvegicus	—	10	KOLLER and DARLINGTON (1934)
" "	—	0	OGUMA (1935)
" *rattus*	—	0	" (")

* E indicates proportion of the equational separation. In *Triticum vulgare*, *Mecostethus gracilis* and *Trimerotropis crinita* more than two cases of heteromorphic pairs are known. Proportions in those different pairs are distinguished by alphabet or numeral in parentheses annexed to the species name. The data annexed with (MI) and (AI) indicate those obtained at first metaphase and at first anaphase respectively, thus the former presenting the proportion of the equational metaphase configuration and the latter that of the equational anaphase separation. In the other data stages were not treated separately or anything was remarked about them. Observed numbers put in parentheses show the number which was given in relation to another problem not directly related to the question here concerned.

Note: There are some controversies as to the number, structure and behavior of sex chromosomes in *Muridae* (cf. OGUMA 1934, 1935, 1937, MAKINO 1941). Further, WITSCHI's observation on *Rana temporaria* was rendered doubtful by the critical investigation of MAKINO(1932). But, apparently, these affect in no way the discussion in the text. (cf. Postscript in p. 90)

speaking, the possibility of universal occurrence of the asynapsis of the kinetochore regions in the whole nuclei can not be doubted. The writer is inclined to believe the universal asynapsis of the kinetochores to be the mode of chromosome pairing in the majority of cases where the kinetochores appear usually separated before first metaphase. For it is to be supposed that the prophase proceeds so rapidly that the kinetochore maintains its mitotic nature until the diplotene condition is completed throughout the nucleus. For example, in *Paris* and *Trillium*, in which kinetochores appear synapsed at metaphase, meiosis is a process prolonged for a few months, while, in *Triticum, Secale* and other plants, in which kinetochores are in separated condition at metaphase, it is completed in a few days or weeks.

Now we are to consider the diversity in the proportions of equational and reductional first anaphase separations of the heteromorphic chromosome pairs, which vary nearly from OE:100R to 100E:OR, in percentage ratio, in a series of different organisms (Table 30). First the problem will be dealt with, letting aside the effect of crossing over. When the kinetochores of the homologous chromosomes in question are always asynaptic, separation should be invariably reductional at first anaphase, that is, 100 per cent prereduction. This asynapsis will be brought about facultatively by the physiological process of the meiosis just as mentioned above or obligatorily by the structural difference between the partner chromosomes. Most of the cases where first anaphase is always reductional, especially the majority of the sex-chromosomes, seem to belong to this category. Indeed obligatory prereduction due to structural differentiation is inferable in many cases of sex-chromosome in plants and animals (cf. DARLINGTON 1937, p. 365). If kinetochores synapse always effectively, then the ratio is expected to range between 66.7E:33.3R and 88.9E:11.1R, in percentage, depending upon the grade of interference of the closed arm loop to the random assortment into a pair of two of the four daughter kinetochores (cf. p. 23). The frequency of asynapsis of the kinetochores will be variable in different organisms and even in the same organism under different environmental as well as genetical conditions[1]. Variation in this frequency will lead to

1) Asynapsis of the kinetochores happens very rarely in *Paris verticillata*. In other plants the prophase separation of the kinetochores is not decisive whether it results from asynapsis or desynapsis. If we assume that the kinetochore separation before first metaphase originates in asynapsis, there are found some supports for the present inference as to the variability in the frequency of asynapsis of the kinetochores. Namely, in *Diphylleia Grayi* the prophase separation of the kinetochores occurs in the majority of bivalents in contrast to the case of *Paris verticillata* (SOEDA 1942). The same holds true also in *Tradescantia* spp., metaphase pairing of the (*continued to the next page*)

the continuous seriation of the proportions of pre- and postreduction from OE:100R to 88.9E:11.1R in percentage ratio. Thus the proportions within the above range are readily explicable without considering the effect of crossing over.

However, the above interpretation fails completely to account for the proportion of postreduction exceeding 88.9 per cent. But, in reality such proportions are known, though some of the data and statements seem rather unreliable (Table 30)[1]. The proportion of postreduction of the range 88.9–100 per cent will be understood only introducing the effect of crossing over in the foregoing considerations. Crossing over on this occasion implies nothing other than the fact that it results in recombination between two non-sister chromatids, disregarding the mode and time of its occurrence. For example, 100 per cent postreduction should take place when the kinetochores are always asynaptic and one crossing over happens invariably in the equal segment between kinetochore and differential segment, the length of this synaptic equal segment being thus 50 genetical units. Then, if kinetochores are invariably asynaptic, variation in the frequency of crossing over in that equal segment is responsible for the whole range of variation in the proportions of pre- and postreduction. This is the interpretation given by MATHER (1935c) to account for the diversity in the proportions of pre- and postreduction in different cases, in his deduction chiasma being equivalent to crossing over. However, it must be especially emphasized that his interpretation rests entirely on the prime presumption that the kinetochores always separate reductionally. As repeatedly pointed out, this assumption is not universal. Furthermore it must be remembered that the interpretation last mentioned has been proved to be completely defective to explain the facts established with the heteromorphic bivalent DD- of *Paris verticillata* (cf. p. 21). Of course, in view of the well established fact that the recombination between non-sister chromatids must result from crossing over, the simple generalization of the preceding interpretation is

(*continued*) kinetochores appearing in about 7 per cent of the cases (SOEDA 1943). These, probably, represent the cases of variation between different organisms. It has been revealed in *Trillium kamtschaticum* that the frequency of prophase separation of the kinetochores is greatly enhanced by the influence of high temperature (MATSUURA and HAGA 1942). This is the variation due to environmental condition. Finally, if the opinion is allowed that the so-called localized chiasma represents in reality the synapsed kinetochores, the following case suggests, perhaps, genetical control of the pairing of the kinetochores. Chiasma in *Allium fistulosum* is of so-called localized formation and that in *Allium Cepa* is of random formation. Their F_1 hybrids show random distribution of chiasmata (EMSWELLER and JONES 1934, 1935, LEVAN 1936, MAEDA 1937, 1942).

1) cf. Genetical data in *Ustilago* spp. (HÜTTIG 1931, 1933a, b).

also to be subjected to a certain limitation.

Consequently it will be safely concluded as follows: Since there is no ground to believe a priori in the total prereduction of the effectively synapsed kinetochores, variation in the frequency of asynapsis of the kinetochore region must play the prime rôle in variation in the proportion of pre- and postreduction, the effect of crossing over and interference to kinetochore assortment of the loop of closed arm configuration or of secondary chiasma being subsidiary to that of the former.

In this connection it will be worth mentioning some remarks as to the genetical significance of the foregoing considerations. The classical problem of pre- and postreduction is now rendered meaningless so long as it concerns the entire chromosome or whole nucleus. For, that the first anaphase separation of a definite portion or locus of a chromosome pair is either equational or reductional is now well established cytologically as well as genetically (cf. GOLDSCHMIDT 1932, BRIEGER 1933). Furthermore this will be quite convincing if we take in consideration the effect of crossing over, whatever its mechanism may be. Therefore the meaning of pre- and postreduction is to be confined, critically saying, only to a definite region or locus of the chromosome. On this reason the cytological behavior of a differential segment of heteromorphic chromosome pairs offers elucidation for some genetic behavior of an allel. For instance, HÜTTIG (1931, 1933a, b) performing tetrad analysis with *Ustilago* species, found the interesting fact that the proportion of pre- and postreduction of the sex factor may be altered by changes in temperature and under the influence of various chemical agents. As already mentioned, frequency of asynapsis of kinetochore region is much influenced by temperature (cf. foot-note in p. 70). Further frequency of crossing over is known to be altered by temperature and other agents (cf. RECK 1936). Therefore alteration in one or both of these frequencies will be, probably, responsible for the observed alteration in the proportion of two modes of separation of the sex factor.

Next, the cause and consequence of chiasma formation will be considered. On the chiasmatype theory chiasmata are formed originally always interstitially as a result of crossing over between two non-sister chromatids of the four involved. And the originally interstitial chiasmata can move towards the distal end by terminalization, resulting in terminal chiasmata (cf. DARLINGTON 1937). Therefore chiasma is assumed as a cytological consequence of genetical crossing over. Here it is emphatically pointed out that this theory rests entirely on the unproved prime assumption, since the time of JANSSENS (1909), that the diplotene opening out

separates the partner chromosomes so that the pairs of chromatids which remain together after the formation of chiasmata are sisters, derived from the same parent chromosome. In reality, however, this assumption was proved to be incorrect with the statistical data in *Paris verticillata* and *Trillium kamtschaticum* (the present study, MATSUURA 1937a, 1938). The statistical data in these plants show indisputably that the diplotene opening out occurs at random either equationally or reductionally with the ratio 2E:1R, which is predicted on randomness in assortment into pairs of two chromatids of the four chromatids. This is the fundamental basis which afforded the formulation of the neo-two-plane theory.

Therefore it is justified to consider the interstitial chiasmata to be formed by the meeting of openings out originated in different modes of opening out. This view cannot be rejected as far as crossing over does not affect the mode of opening out, even if crossing over takes place before or at the same time of diplotene opening as usually supposed. Thus chiasmata are formed in their origin with no relation to crossing over. Then, as already analysed, two independent factors are distinguishable, which are responsible for the formation of interstitial chiasmata. The first is the length factor, that is, the primary interference, which conditions the number of openings out and is inherent to chromosome length itself. The second is the time factor, that is, the secondary interference, which conditions the time between the openings out which may occur in succession. The latter is also correlated with the chromosome length but varies under different environmental conditions. These two factors are entirely independent on each other, the former concerning only the space and the latter only the time. And these two factors determine in co-operation the chiasma frequency. Consequently chiasma frequency is expressed as a function of square of chromosome length as really ascertained. If the time factor becomes negligible, that is, if the diplotene opening out is completed simultaneously throughout the chromosome length, the chromosome length-chiasma frequency relation will approach the linear rather than the exponential expression. The condition just mentioned above implies the rapid completion of diplotene process, perhaps, accompanied by the general acceleration of the whole process of meiosis. This modified relation was really revealed in plants with high chiasma frequencies, which suggest the high velocity of diplotene completion. In reality chiasma frequency is proportional to arm length when the kinetochores remain synapsed until the first metaphase, and to entire chromosome length when the kinetochores are in separated condition either asynaptically or desynaptically before

or at the time of opening out. The former condition is due to the physical nature of synapsed kinetochores, which divide the chromosome mechanically into two independent portions.

The most interesting point revealed in the foregoing analyses will be compressed in the following two facts. The first is that the maximum number of interstitial chiasmata to be formed in a given chromosome or organism is constant, being determined by the length factor. In other words, maximum mean chiasma frequency is inherent in any organism determined by its own constant distance of primary interference in opening out, being attained when the effect of secondary interference in opening out is nullified. Accordingly, vast majority of cases of variation in chiasma frequency in an organism is due to variation in the grade of secondary interference, which may be altered under different environmental conditions. The second interesting point consists in that chromosome length-chiasma frequency relation follows quite independent systems in three different length classes, which are able to form, at maximum, one, two and more than three chiasmata respectively. This fact is important in revealing the specificity of the length as a whole in chiasma formation. In view of these results it seems to be advisable to reinvestigate critically the previous data on chromosome length-chiasma frequency relation. In this reinvestigation it is needed to pay special attention to the behavior of kinetochores and to treat separately, at least in the first place, the terminal chiasmata from the interstitial ones.

The effect of terminalization of interstitial chiasmata was not taken into account in the foregoing consideration. Thus, here, recent opinions on this problem of terminalization will be mentioned. MATHER (1940) stated from his analysis of the position of chiasmata as follows: "Thus, in general, terminalization is all or none process. Either the chiasmata all terminalize in a bivalent or, at most, only the most distal ones move to the ends, there being no reduction in chiasma frequency". The intermediate terminalization may take place by the occurrence of the above two alternative behaviors in different bivalents in a nucleus or by the arrestment of the terminalization movement by the later stages of meiosis commenced before it can be completed. However, "such cases may be expected to be rare" (p. 222). This conclusion seems to be very suggestive though his method of analysis rests fundamentally on the questionable assumption that "chiasmata do not occur haphazardly along the bivalents but arise in certain definite regions, most probably related in position to the centromere" (p. 206).[1]

1) This conclusion of MATHER was reached originally (*continued to the next page*)

Recently NEWCOMBE (1941) has also offered an opinion as to this problem. He emphasized the fact "that different chiasma pairs observed by HUSKINS and NEWCOMBE (1941) tend to be of different length, whereas, were there an appreciable rearrangement due to repulsion between chiasmata, all the simpler types would tend to be of the same length" (p. 135).

In *Paris verticillata* and *Trillium kamtschaticum* there are decisive evidences against the terminalization of interstitial chiasmata. Terminalization necessarily follows the separation of the paired chromatids, which is restored by the new association of two chromatids not associated prior to terminalization. If this happens in *Paris verticillata,* most of the exceptional bivalent DD^-, which are held together only by the unmated differential segment satellite, would be separated into two univalents by the separation of two paired chromatids of satellite under the influence of repulsion between kinetochores, resulting in a pair of equally shaped univalents. But this is not the case, pairs of univalents being invariably heteromorphic as to the presence and absence of the satellite. The discovery of the secondary chiasma in *Trillium kamtschaticum* is very significant in connection with the present problem (MATSUURA 1941b). Specific chiasmata with very small loop or distal portion were observed frequently in this plant either just next to the synapsed kinetochores, interstitially adjoining the ordinary larger loops or distally close to the terminal end. In all probability the formation of these specific chiasmata can not be explained by chiasmatype theory which maintains the chiasma interference equivalent with the interference in crossing over. On the contrary, the frequency of formation of these specific chiasmata is found to conform with that expectable from the principle of neo-two-plane theory. From these facts MATSUURA (l. c.) has concluded as follows: "It is evident that the present findings and interpretations are at variance with the following hypotheses of DARLINGTON and his school, (i) of chiasma terminalisation, (ii) of chiasma interference in relation to crossing over interference and (iii) of terminal affinity (cf. DARLINGTON '37)" (p. 386). Therefore it is unquestionable that the terminalization does not occur at all in *Paris verticillata* and *Trillium kamtschaticum*. This offers the fundamental basis for the foregoing deduction of the chromosome length-chiasma frequency rela-

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(*continued*) by the analysis of the genetical data in *Drosophila melanogaster* (MATHER 1936b). But it was rendered questionable by CHALES (1938) on the ground of the similar study on X-chromosome of the same fly. The latter worker mentions as follows: "In so far as each step in MATHER's argument is at least uncertain in the light of the present data, the ultimate conclusion would not necessarily seem to follow" (p. 123). Further see BOOST (1939).

tions. In many other organisms the same may hold true likewise. Then the terminal chiasmata must be developed in their origin without causal relation to the interstitial ones. Thus it is quite conceivable that the terminal chiasma is the terminal junction developed by opening out, which has ceased its development at the terminal end, and maintained by the viscous matrix, at least in organisms such as *Paris verticillata* and *Trillium kamtschaticum*. This interpretation conforms well with the several facts revealed in the present study, as pointed out in the item concerning the terminal chiasma.

In spite of the above cases it seems undoubtful that the terminalization really occurs in certain other organisms, for the actual number of terminal chiasmata, not the terminalization coefficient, increases with the progression in stages accompanied by the reduction in actual number of interstitial ones (cf. Table 73 in DARLINGTON 1937, UPCOTT 1936, MAEDA 1939, etc.). Reduction in the number of interstitial chiasmata, not accompanied by the increase in the number of terminal chiasmata, can also be explicable alternatively by the SAX's theory of crossing over (SAX 1930, 1932)[1]. He postulates "breaks in the chiasmata so that a decrease in the number of chiasmata would be expected between early diplotene and metaphase if crossing over occurs" by the break and reunion of chromatids at the chiasmata which are formed following the classical two-plane theory (cf. SAX 1932, p. 192). But his interpretation fails to account for how the number of terminal chiasmata is really increased. No other theories than that of terminalization seem to be able to explain the above fact that the number of terminal chiasmata increases from earlier to later stage. In this connection it is very interesting that terminalization in *Campanula persicifolia* can be retarded under the effect of high temperature, many interstitial chiasmata remaining still interstitially at metaphase (STRAUB 1936). Otherwise, this plant is one of those characterized by the complete terminalization, showing only terminal chiasmata at metaphase (GAIRDNER and DARLINGTON 1931). Under these circumstances MATHER's final conclusion quoted above seems to be valid, in so far as the present knowledge of so-called terminalization phenomenon indicates. Thus it remains for further critical investigations as an interesting and important cytological problem to elucidate the phenomenon of terminalization, especially in relation to the behavior of the spiralized chromonemata.

According to the chiasma theory of pairing, the homologous chromo-

1) This theory of crossing over has been discarded by SAX himself (cf. SAX 1936).

somes are held together, after diplotene stage, merely by virtue of chiasmata which may or may not terminalize (cf. DARLINGTON 1937). However, this hypothesis is objectionable to be general because, as it has been repeatedly emphasized, synapsed kinetochores play a definitive rôle in chromosome pairing in certain organisms such as *Paris* and *Trillium* species (cf. MATSUURA 1941a). Therefore the pairing can be maintained by one of the following three means, that is, synapsed kinetochores, interstitial chiasma and terminal chiasma, the last being supposed to be derived directly from the diplotene opening out which ceased its development at the terminal end, or possibly from the terminalization of interstitial chiasmata as mentioned above. Only when the kinetochores are asynaptic or desynaptic, the pairing of the homologous chromosomes owes solely to the formation of a chiasma or chiasmata which may be either interstitial or terminal. Hence the non-pairing at metaphase is expected to be inversely functional to the formation of chiasmata which is in turn functional to chromosome length. In fact this relation was established in several cases as already shown. Of course asynapsis, possibly also desynapsis, of the kinetochore region is prerequisite in this process of univalent formation. Early prophase separation, at least asynapsis, of the kinetochores happens accidentally in some of chromosome pairs or habitually in all chromosome pairs in the nuclei, probably with no relation to the chromosome length[1]. Thus the relative frequency of non-pairing of the chromosomes of different lengths is determined by the chiasma frequency.

It is well known that non-pairing is caused under the influence of high or low temperature (SHIMOTOMAI 1927, TAKAGI 1928, HEILBORN 1930, NAKAMURA 1936, STRAUB 1936, 1937, etc.), of water content of the plant body (STRAUB 1937), of age or 'daily condition' (KIHARA 1929, HOLLINGSHEAD 1932, MATHER 1935b, etc.) and of other conditions. However, regretfully it seems as yet not certain in what manner three means of the chromosome pairing are affected by the agents above mentioned. Rapid transition from resting to meiotic stage will cause the asynapsis of the kinetochore and its neighboring regions as already suggested. If this asynaptic region converts into diplotene condition much prior to the synapsed region and leads up forcibly the opening out of the synapsed region under those circumstances, the formation of interstitial as well as terminal chiasmata would be inhibited in certain appreciable amount, result-

1) However, rather astonishingly, frequency of the prophase separation of the kinetochores in *Trillium kamtschaticum* indicates a tendency of its decrease with chromosome length (cf. Table 14). Thus this point remains for further investigations.

ing non-pairing at later stages. This interpretation is supported by the facts established in the present study. Complete asynapsis in the whole nuclei is apparently due to another cause, that is, the alteration of the system of division from meiotic to mitotic (MATSUURA and HAGA 1940).

Finally some critique will be made as to the so-called evidences for chiasmatype theory. On this theory of crossing over, chiasma is raised as a consequence of crossing over, diplotene opening out being assumed to take place always in reductional manner. It seems generally accepted by the recent cytologists as well as geneticists that the decided evidences for this theory are afforded by the following so-called critical configurations (cf. DARLINGTON 1937, MATHER 1938).[1] (1) Double interlocking of the two non-homologous bivalents (MATHER 1933, 1935a, BEAL 1936, UPCOTT 1936), (2) "figure-of-eight" brought about by a chiasma formed between the two points of interchanges in double interchange heterozygote (DARLINGTON 1931, SANSOME 1932), (3) multivalent pairing where one chromosome forms a chiasma with a second member between two chiasmata which it has formed with a third (DARLINGTON 1930, DARLINGTON and MATHER 1932, etc.) and (4) pairing of a fragment by an interstitial chiasma with a major bivalent (MATHER 1935a). However, all of these evidences can hardly be considered as decisive because there remain more reasonable interpretations based on neo-two-plane theory.

Genetically it is well established that the crossing over occurs in a four strand stage, and it involves only two of the four strands at any point of interchange. So assume that crossing over happens at early diplotene before opening out is commenced, but that crossing over does not interfere with the mode of opening out of the four chromatids into pairs of paired chromatids, random assortment of chromatids taking place in opening out, following the neo-two-plane scheme. According to this consideration, crossing over and chiasma formation are the independent phenomena with no causal relationship.

Then so-called cytological evidences for chiasmatype theory can be fully interpreted as specific modified cases on the ground of neo-two-plane theory. First, points of imprisonment in the double interlocking configuration are asynaptic mechanically at pachytene; two adjacent loops of a

1) In every case of the so-called critical observations, if we assume a second chiasma, which has broken off, as SAX's hypothesis of crossing over demands, all the observations may become in accordance with the classical as well as neo-two-plane theory of chiasma formation (cf. SAX 1932, MATHER 1938). But such interpretation seems to be highly improbable.

bivalent, through which two sides of a loop of the second bivalent are interlocked, are thus necessarily to open out reductionally, usually, much prior to the opening out of the synapsed region between the points of imprisonment. This inference is supported by the behavior of the asynaptic region in the case of univalent formation. Then, if crossing over really occurred in that region between the two points of imprisonment, there should be formed a chiasma. "Median chiasma" in "X segment" of the association of six chromosomes should result from the crossing over in X segment between two points of segmental interchange. As is evident in many cases of segmental interchange heterozygotes, the points of segmental interchange open out always reductionally prior to the opening out of neighboring regions, otherwise ring or chain configuration becoming unexpectable. This predetermined reductional opening out is readily comprehensible, as the point of segmental interchange may be comparable with the asynaptic point in respect to the pairing relationships of the chromosomes. Hence the X segment is to be opened out under the influence of the openings out of the two points of segmental interchange, which occur much prior to that of the synapsed X segment. Therefore median chiasma is formed if crossing over has happened in the stage before diplotene opening out commences. Specific configurations of the multivalent pairings are likewise explicable in similar manner. These configurations, except the pairing of a fragment with an interstitial chiasma, are derived possibly from the pairing involving, at least, one of the two distal regions of all three or more chromosomes at pachytene, diplotene opening out being followed by random assortment of the daughter chromatids into pairs of paired chromatids.[1] Such a pairing of all three chromosomes is really observed, though partially, in *Nicotiana tabacum* (OLMO 1934) and *Lilium tigrinum* (CHANDLER, PORTERFIELD and STOUT 1937). Thus the so-called critical cytological evidences for chiasmatype theory are interpreted alternatively and more reasonably as the modified cases of neo-two-plane scheme of opening out. Consequently no reason is found to believe those evidences to be in favor of chiasmatype theory of crossing over.[2]

1) This interpretation seems, however, difficult to apply universally, for this manner of formation is to result frequently in interstitial triple chiasma which is not known as yet.

2) DARLINGTON mentions as a cytological evidence of crossing over the particular cases of relational coiling around the paired diplotene chromatids (cf. DARLINGTON 1936b). However, according to the opinion of SAX, "most of DARLINGTON's observations" regarding relational coiling are incorrect (SAX 1936, p. 336). At any rate his evidence can not be taken only for chiasmatype theory, an alternative interpretation being equally possible with the assumption postulated (*continued to the next page*)

Another source of so-called evidences for chiasmatype theory of crossing over is supplied from the comparison between cytological observations and genetical analyses (cf. MATHER and LAMM 1935, DARLINGTON 1937, MATHER 1938). However, most of the emphasized parallelisms are plainly understandable, considering the fact that chiasma formation and crossing over are both conditioned by the chromosome pairing, though there is no causal relationship between those two phenomena as postulated above. The interesting case on this line is the absence of chiasma formation in male *Drosophila* species and femal *Bombyx mori* which is paralleled by the non-occurence of crossing over in the same sex of these insects. The autosome bivalents of *Drosophila pseudoobscura* are found to "consist of four chromatids equally paired throughout their length without chiasmata, a condition made possible by exaggerated somatic pairing and changed precocity" (DARLINGTON 1934, p. 115). All the bivalents of female *Bombyx mori* are paired only by a terminal chiasma at one end from the stage as early as diplotene; this terminal junction probably does not represent the synapsed kinetochore, because in the male there were found bivalents provided with two terminal chiasmata in 16 out of 100 bivalents proving that true terminal chiasma is formed at least in certain bivalents of the male and that, at least, in certain chromosome pairs the location of kinetochores is not terminal (MAEDA 1939). Therefore there remain many possibilities that certain physiological conditions which have brought about these anomalous pairing have simultaneously prevented the crossing over.

Thus it will be concluded that all of the so-called evidences are not apt to prove the validity of chiasmatype theory. Furthermore, statistical evidences, from various sources, for neo-two-plane theory of bivalent constitution and of chiasma formation can be explained by no means from the viewpoint of chiasmatype theory, even under the speculative modifications of its prime presumptions that the diplotene opening out takes place invariably in reductional manner and that the chiasmata do result as a consequence of preceding crossing over.

On the other hand, the "critical configurations" mentioned above are impossible or hardly to interpret by the simple application of the view of classical or neo-two-plane theory of chiasma formation. This argument will be quite convincing in the frequency of "figure-of-eight" in the double interchange heterozygote in *Pisum sativum* as maintained by the school

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(*continued*) in the present paper.

Further, so-called inversion-bridge configuration can never be convincing as an evidence for that theory (cf. the second paper of this series).

of chiasmatype theory, the frequency attaining to about 78 per cent, that is, 62 out of 78 configurations (SANSOME 1932). This frequency strongly rejects the doubt of the misinterpretation of the configuration. The case of double interlocking points to the same conclusion, for example the "critical chiasma" in the case of *Eremurus spectabilis*, is formed in a median position of a interlocked bivalent, removing the alternative interpretation that the position of critical chiasma represents the synapsed kinetochore because all the chromosomes of this plant are provided with subterminal kinetochores (UPCOTT 1936). In the second case the position of critical chiasma may represent the point of sporadic break and reunion of chromatids; but such interpretation seems hardly to be possible. In this way simple adoptation of the classical as well as neo-two-plane theory fails to account for these particular cases of chiasma formation.

However, these opposing evidences, one for neo-two-plane theory and the other for chiasmatype theory, are completely reconciled with the following postulation. That is, crossing over happens at diplotene before opening out commences, and chiasma is formed by meeting of diplotene openings out developed in different modes of opening out, the latter taking place in no causal relation to the preceding crossing over, following the scheme of neo-two-plane theory.[1] Further, subordinately, a chiasma is formed even when the mode of two adjacent openings out is identical if there has occurred a crossing over between those two openings out, and, contrariwise, crossing over cancels certain proportions of possible chiasma which might be realized as a chiasma if there happened no crossing over.[2] Of course this is a speculation still open to question. Whatever may be the validity

1) This postulation does not conform with the theory of crossing over put forward by MATSUURA (1940) with regard to the time and mode of crossing over.

2) Occurrence of a crossing over, anterior to opening out, between two points of openings out does not affect the probability of formation of a chiasma, the probability remaining still 2/3 as in the case involving no crossing over. This relationship holds true, in so far as the distance of interference in crossing over is not so small that the double crossing over does not occur within the distance of primary interference in opening out. Thus, seemingly, the effect of crossing over to chiasma frequency is subsidiary to that of opening out which occurs following the neo-two-plane scheme. The only influence of crossing over to chiasma frequency is that, if the frequency of the former is enhanced, that of the latter tends likewise to be enhanced. Therefore the effect of crossing over to chiasma frequency is just the same as that of secondary chiasmata. In the foregoing analysis of the relation of chromosome length to chiasma frequency these effects of secondary chiasma and crossing over were neglected to make simple the deduction. Fuller discussion on these points will be made on later occasions elsewhere.

of the above postulation, it will be concluded that there is too little fact or evidence to believe a priori the foundation of chiasmatype theory.

Concluding the present study, it will be worth mentioning that interference occurs both in chiasma formation and crossing over (HALDANE 1931), in the former two constituents, primary and secondary interference, being distinguished by the present investigation. Comparison of the critical properties between the interferences in chiasma formation and in crossing over is of prime importance in this line of investigation. But, regretfully, there are no cytological data as yet which could be apt to be compared with the genetical data such as presented by BOOST (1939). This comparison will be probably made possible by the statistical analysis of the position of chiasmata.

Summary[1)]

A diploid karyotype 2n–III of *Paris verticillata* (2n=10) was employed throughout the present study. This karyotype is characterized by the heterozygous condition as to the presence and absence of a satellite of D type chromosome pair. This peculiarity rendered possible to make critical analyses of the several interesting phenomena. Further, in this plant, generally speaking, kinetochores of the homologous chromosomes remain synapsed until the first anaphase commences. Important findings in the present study are to be mentioned as follows:

1. The mode of chromatid opening out at diplotene is either equational or reductional, following the neo-two-plane scheme. This was proved statistically employing the heteromorphic bivalent DD^-. Really the ratio between the frequencies of equational and reductional openings out was found to be 667 equational to 333 reductional in a total of 1000 heteromorphic bivalents, that is almost exactly 2:1 ratio, in accordance with the expectation from the principle of MATSUURA's neo-two-plane theory. This ratio is obviously brought about by the random occurrence of three modes of opening out, two different equational and one reductional mode of opening out taking place with equal chance.

2. Accordingly, the prime presumption of chiasmatype theory, which demands the diplotene opening out to happen invariably in reductional manner, was indisputably rejected on the ground of a good evidence. Therefore it is justified that the interstitial chiasma is formed by the meeting of openings out which were developed in different manner of

1) The term chiasma in this summary implies only interstitial chiasma, terminal chiasma being distinguished from it by always adjoining the adjective terminal.

opening out. Since there are three modes of opening out, the probability of differing of the mode of opening out between the two adjacent openings out is 2/3.

3. Statistics of the chiasma frequencies of the individual chromosomes revealed that the increase or reduction in the mean chiasma frequency does not result from the increase or reduction in the maximum number of chiasmata formed in individual chromosomes, the maximum number remaining constant even in the cases of strikingly divergent mean chiasma frequencies. Thus the high chiasma frequency is brought about by enhancing the chance of chiasma formation, not of the maximum number possible in the individual chromosomes. The constancy of the maximum number of chiasmata to be formed in a given chromosome is obviously due to the constancy of the minimum length of opening out; this minimum was termed as primary interference in opening out. Repulsion force between chromatid pairs in already opened region forces to open out the adjoining unopened regions, thus the opening out proceeds further beyond the distance of primary interference. Interference in this manner was termed as secondary interference. This second type of interference was substantiated, though indirectly, by the first anaphase behavior of the heteromorphic pairs.

4. Opening out is initiated at any possible position, but happens as a block—primary interference. Hence in the arms of the length within the length of primary interference no interstitial chiasma is formed. Beyond this minimum length, the possibility of the occurrence of the second opening out is increased proportionally to the length. This implies the proportionality of the chiasma frequency *spatially* to the chromosome length. Secondary interference reduces the chance of occurring of the second opening out. This effect of secondary interference is reduced with the increase in length. Thus, increase in length enhances the chance of occurring of the second opening out and so on *in timing relation* between the openings out which may occur in succession. The former was called length factor and the latter time factor in chiasma formation. Both factors are correlated with the length, but are of entirely independent nature. Therefore chiasma frequency is proportional to the square of length. This chromosome length-chiasma frequency relation was fully substantiated with the data observed.

5. When the effect of time factor becomes a nullifiable grade, the chiasma frequency expresses rather simple linear function of the length than the function of square of length. This is the natural consequence

from the relation just above mentioned and was confirmed with the data in plants showing high chiasma frequency, high frequency of chiasma formation representing an indication of the rapid progression of the meiotic process.

6. Frequency of interstitial chiasmata correlates with the arm length, when the kinetochores remain synapsed until first anaphase. This is apparently due to the effect of the synapsed kinetochores which divide the chromosome mechanically into two independent portions. If the kinetochores are in separated condition from the early prophase stage, asynaptically or desynaptically, the frequency of interstitial chiasmata represents a function of the entire length. These relationships were also ascertained with the actual data.

7. In *Paris* and *Trillium* chiasmata apparently do not move after the time of formation. Evidences for this conclusion were offered from several observations. Accordingly, terminal chiasma is considered to be formed, in its origin, in no causal relation to the interstitial chiasmata. Terminal chiasma probably originates from the opening out which ceased its development at the distal end, representing the connection by virtue of viscous matrix. Frequency of terminal chiasmata correlates positively with the chromosome length and negatively to the formation of interstitial chiasmata. These relations were explained taking in consideration the repulsion force between the chromatid pairs.

8. Chromosome pairing is maintained at least by one of the following three agents, that is, synapsed kinetochores, interstitial chiasma and terminal chiasma. Accordingly, accidental univalent formation occurs when the kinetochores are separated and no chiasma, interstitial as well as terminal, was formed. Thus, frequency of univalent formation is expressed as an inverse function of the chiasma frequency or chromosome length. This was ascertained with several data from various sources. Further, in *Paris verticillata* it was revealed as an interesting fact that the univalent formation is raised by the asynapsis of the kinetochore and its neighboring regions followed by complete desynapsis of the distal regions.

9. It was verified that there is no significant correlation or competition between the bivalents in chiasma formation. In this specific analysis there were revealed the following important facts. Namely, increase in the mean chiasma frequency is accompanied by reduction of the variation in the number of chiasmata formed in the nuclei. This is due to the fact that approaching to the maximum mean chiasma frequency reduces the extent of the variation of the number of chiasmata to be formed, the theoretical

maximum mean chiasma frequency per nucleus being calculated by the use of the value of primary interference as 7.82 for *Paris verticillata*. And the sign of the calculated correlation coefficient changes from positive to negative at the middle level of the whole range of variation in the mean chiasma frequencies. This is the fact revealed by the present study, not known previously, and is readily explained as a mechanical consequence from the relation between the mean chiasma frequency and the variation in the number of chiasmata formed in the nuclei.

10. Meiotic behavior of the heteromorphic chromosome pairs was reviewed at some length. Variation in the proportion of pre- and post-reduction of the heteromorphic pairs varies nearly from perfect prereduction to perfect postreduction in a series of various cases. Frequency of asynapsis of the kinetochore region was pointed out to be of prime importance in determining the proportion of the two modes of separation, the effect of crossing over and of interference of the closed arm loop or secondary chiasma loop to the kinetochore separation being subsidiary to that of the former.

11. A critique was made as to the so-called evidences for the chiasma-type theory and it was shown that all the evidences are not decisive for that theory. Indeed, alternative explanation was successfully given on the ground of the neo-two-plane scheme of opening out and of chiasma formation.

12. Finally, the followings were given as the conclusion from the present study. Opening out at diplotene of the effectively synapsed region follows the neo-two-plane scheme, two different equational and one reductional mode of opening out occurring with equal chance, however, excepting the particular cases such as the region involving the interchange point in translocation heterozygote and the regions neighboring to the imprisonment in interlocking. In the latter cases opening out is always reductional. Thus, the chiasma is formed generally by the difference in the modes of opening out between two adjacent openings out without causal relation to crossing over. Consequently it is more reasonable to consider that chiasma formation and the crossing over are entirely independent phenomena, in so far as the present knowledge indicates. Resemblances between these two phenomena, chiasma formation and crossing over, are of superficial nature, being brought about by the fact that both phenomena are conditioned by the meiotic pairing of the homologous chromosomes.

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Postscript. After the manuscript of the present paper was sent to press, a recent publication by S. MAKINO* became accessible to the writer. In it, he reports that sex-chromosome pair X–Y undergoes invariably prereduction in five species of rats, i.e., OE:152R in *Rattus norvegicus* and OE:164R in *R. rattus*. Postreduction of X–Y pair does not occur also in *R. losea, R. fulvescens* and *R. confucianus*; however, statistical data for these three species were not given.

* MAKINO, S. 1943. Studies on the murine chromosomes. III. A comparative study of chromosomes in five species of *Rattus*. Journ. Fac. Sci. Hokkaido Imp. Univ. Ser. VI Zool. **9**: 19–57.