Chromosome Studies on *Trillium kamtschaticum* Pall.
VII. Additional evidence for the Neo-two-plane
theory of bivalent constitution

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From a statistical study on the mode of chromatid disjunction at the first meiotic metaphase in the PMC of *Trillium kamtschaticum*, the writer ('37b) has given a new interpretation of bivalent constitution which is built upon the following three general principles: (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 the 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.

The above interpretation of bivalent constitution may be called "Neo-two-plane theory" (Matsuura '37a), because it accepts the mode of chromatid opening on the two planes, reductional and equational, as the classical or former two-plane theory claims, as opposed to the chiasmatype or one-plane theory, but is distinguished in the mode of kinetochore disjunction from these former interpretations which are based on the assumption that the associated kinetochores disjoin only reductionally at the first meiotic division.

Based on the present theory, the constitution of cruciferous metaphase bivalents of this material is expected to be such as illustrated by the following diagram (Diagram 1). In this, α , β and γ represent the three possible modes of chromatid assortment, reductional (in α) and equational (in β and γ), within a pair of homologous arms. Consequently with respect to the entire bivalent, one gets the nine possible combinations, of which $\alpha\alpha$, $\beta\beta$ and $\gamma\gamma$ result in the parallel association as previously designated, and $\alpha\beta$, $\beta\gamma$, $\gamma\alpha$ and their reciprocals result in the cross-association. The separation of the four daughter kinetochores takes place in the three possible modes as shown in the upper right of the diagram, one reductional

and two equational, and these combined with each bivalent type are to give the four modes of reduction, E-E, E-R, R-E and R-R, in which E and R designate respectively the equational and the reductional divisions in the cytological sense, and the letters before the hyphen "-" refer to the short arm pair and those after the same to the long arm pair. As the corollary, in the grand total, the ratio of EE:ER:RR for complete bivalents should be 4:4:1 and that of E:R for separate pairs of arms should be 2:1. The previous analytical study gave the actual data of 121:113:24 for EE:ER:RR and 464:225 for E:R.

It might be said however that these theoretical ratios do not necessarily demand that the kinetochore separation of the first division occurs at random, but may be likewise interpreted by assuming that it takes place in the reductional way only. But such an assumption conflicts with the general idea of chromatid disjunction that the four daughter chromonema and the four daughter kinetochores always open two-by-two, with the mere difference in timing relationship that the paired chromonemata open at diplotene and the paired kinetochores open at a later stage. Furthermore the data obtained by Dr. Haga of our Laboratories on the division of the heteromorphic pair in *Paris hexaphylla* are very significant in supporting the present hypothesis (*v. infra*).

Diagram 1. Explanation in text.

It will be inferred from the present hypothesis that the bivalents of cross-association should occur twice as often as those of parallel association. These two types of association, abbreviated as *C* and *P* respectively, are easily distinguishable from each other in the precocious tetrads arising from heat treatment (Figs. 1-7, *cf.*

Figs. 1-7. Representing cross- and parallel associations of precocious bivalents in eight complete PMCs ($\times 747$); the distribution of these two modes of association in these figures is as follows:

	<i>C</i>	<i>P</i>		<i>C</i>	<i>P</i>
Fig. 1.	ACE	BD	Fig. 5.	ABCE	D
„ 2.	ABCE	D	„ 6.	E	ABCD
„ 3.	A	BCDE	„ 7a.	ADE	BC
„ 4.	BE	ACD	„ 7b.	ABE	CD

Matsuura '37d).¹⁾ In an earlier paper ('37c), the writer observed in the total 25 tetrads of the precocious type that C:P equalled 17:8.

Table 1. Distribution of 522 complete PMCs with respect to the mode of association of the individual tetrads (A-E) within the complement. (Gothic letters indicate tetrads of cross-association type, *C*, and italics tetrads of parallel association type, *P*.)

Combination	PMCs obs.	Ratio exp.	Ratio obs.	Deviation	P.E.
5 <i>C</i> +0 <i>P</i> : A B C D E	84	—	—	—	—
4 <i>C</i> +1 <i>P</i> {	A B C D E	1	0.6878	-0.3122	
	A B C D E	1	0.9524	-0.0476	
	A B C D E	1	1.1376	+0.1376	
	A B C D E	1	1.0846	+0.0846	
	A B C D E	1	1.1376	+0.1376	
Total	189	5	5.0000	0.0000	±0.0981
3 <i>C</i> +2 <i>P</i> {	A B C D E	1	1.1538	+0.1538	
	A B C D E	1	0.6154	-0.3846	
	A B C D E	1	1.3077	+0.3077	
	A B C D E	1	1.5385	+0.5385	
	A B C D E	1	0.4615	-0.5385	
	A B C D E	1	0.4615	-0.5385	
	A B C D E	1	1.0769	+0.0769	
	A B C D E	1	1.0769	+0.0769	
	A B C D E	1	0.9231	-0.0769	
	A B C D E	1	1.3847	+0.3847	
Total	130	10	10.0000	0.0000	±0.1775
2 <i>C</i> +3 <i>P</i> {	A B C D E	1	1.4634	+0.4634	
	A B C D E	1	0.9756	-0.0244	
	A B C D E	1	0.9756	-0.0244	
	A B C D E	1	1.0976	+0.0976	
	A B C D E	1	1.0976	+0.0976	
	A B C D E	1	0.9756	-0.0244	
	A B C D E	1	0.8537	-0.1463	
	A B C D E	1	1.2196	+0.2196	
	A B C D E	1	0.6096	-0.3904	
	A B C D E	1	0.7317	-0.2683	
	A B C D E	1	0.7317	-0.2683	
Total	82	10	10.0000	0.0000	±0.2235
1 <i>C</i> +4 <i>P</i> {	A B C D E	1	1.4583	+0.4583	
	A B C D E	1	0.6250	-0.3750	
	A B C D E	1	0.6250	-0.3750	
	A B C D E	1	1.2500	+0.2500	
	A B C D E	1	1.0417	+0.0417	
Total	24	5	5.0000	0.0000	±0.2754
0 <i>C</i> +5 <i>P</i> : A B C D E	13	—	—	—	—

1) In the earlier paper, the writer distinguished two types of the precocious tetrad, "a" and "b". But later studies have shown that some of tetrads described at that time as "precocious-a" are merely of a derived form of the parallel association type of "precocious-b". The difference in configuration between *e.g.* two A-tetrads in Figs. 4 and 6 of the present paper is of minor significance, probably due to a slight modification in timing relationship between the kinetochores and the development of the poles of the division. In the present paper, therefore, no demarcation has been made between these two types. An account on the true "precocious-a" type in the original sense which differs in the mode of origin from the "precocious-b" type will be given at another occasion.

Since the confirmation of this ratio is to serve as the direct proof for the random two-plane opening of chromatids, an attempt was undertaken anew to collect data on this subject on a very large scale. A total of 522 complete PMCs of the precocious type were analyzed in acetocarmine preparations as to their individual tetrads within the complement. The whole data are summarized in Table 1 and from it Tables 2-5 are made.

From these Tables, the following may be stated with certainty:

i) The proportion of $C:P$ is exactly 2:1, as expected, for each member of the complement, indicating that the difference in the position of the kinetochore bears no relation to the mode of chromatid opening (Tables 3 and 4).

Table 2. Distribution of 522 complete PMCs with respect to the different combinations of cross- and parallel associations in the complements.

Combination	PMCs obs.	Ratio exp. (based on the 2P:1C ratio)	Ratio obs.	Deviation	P.E.
5C+0P	84	32	39.1034	+7.1034	± 2.4258
4C+1P	189	80	87.9828	+7.9828	± 3.3712
3C+2P	130	80	60.5173	-19.4827	$\pm 3.3712^*$
2C+3P	82	40	38.1724	-1.8276	± 2.6603
1C+4P	24	10	11.1724	+1.1724	± 1.4250
0C+5P	13	1	6.0517	+5.0517	$\pm 0.4593^*$
Total	522	243	243.0000	0.0000	

In cases marked * the deviation surpasses three times the P.E.; this would be ascribed to rather small number of observations.

Table 3. Proportion of cross- and parallel association in the five chromosome members of the complement.

Chromosome	Association	Observed	Ratio exp.	Ratio obs.	Deviation
A	C	346	2	1.9885	-0.0115
	P	176 } 522	1	1.0115	+0.0115
B	C	351	2	2.0172	+0.0172
	P	171 } 522	1	0.9828	-0.0172
C	C	352	2	2.0230	+0.0230
	P	170 } 522	1	0.9770	-0.0230
D	C	353	2	2.0287	+0.0287
	P	169 } 522	1	0.9713	-0.0287
E	C	352	2	2.0230	+0.0230
	P	170 } 522	1	0.9770	-0.0230

For each case, P.E.= ± 0.0418 .

Table 4. Proportion of cross- and parallel associations in the grand total.

Association	Observed	Ratio exp.	Ratio obs.	Deviation
<i>C</i>	1754	2	2.0161	+0.0161
<i>P</i>	856	1	0.9839	-0.0161
Total	2610	3	3.0000	0.0000

P.E. = ± 0.0187 .**Table 5.** Test table for the non-correlation between different chromosome types with respect to the mode of association. α) Cross-association

Combination	Cases obs.	Ratio exp.	Ratio obs.	Deviation
AB	241	1	0.9853	-0.0147
AC	236	1	0.9648	-0.0352
AD	236	1	0.9648	-0.0352
AE	242	1	0.9894	-0.0106
BC	241	1	0.9853	-0.0147
BD	238	1	0.9730	-0.0270
BE	256	1	1.0466	+0.0466
CD	256	1	1.0466	+0.0466
CE	247	1	1.0098	+0.0098
DE	253	1	1.0344	+0.0344
Total	2446	10	10.0000	0.0000

P.E. = ± 0.0409 . β) Parallel association

Combination	Cases obs.	Ratio exp.	Ratio obs.	Deviation
AB	66	1	1.0154	+0.0154
AC	60	1	0.9231	-0.0769
AD	59	1	0.9077	-0.0923
AE	66	1	1.0154	+0.0154
BC	60	1	0.9231	-0.0769
BD	56	1	0.8615	-0.1385
BE	75	1	1.1538	+0.1538
CD	73	1	1.1231	+0.1231
CE	65	1	1.0000	0.0000
DE	70	1	1.0769	+0.0769
Total	650	10	10.0000	0.0000

P.E. = ± 0.0794 .

ii) For the chromatid opening, a tetrad acts as an independent unit, no relation existing between any two different members of a complement (Table 5).

It is clear then that the whole data support the present theory in a remarkable fashion.

Heretofore concerning the problem of chromatid disjunction in meiosis, the behavior of unequal bivalents was usually relied upon

as offering direct evidence, and very diverse conclusions have been reached by various investigators in different organisms. It must be mentioned however that caution is necessary in applying their data directly to normal bivalents because of possible differences of heteromorphic from normal bivalents with respect to the mode of chromatid disjunction.

The chiasmotype theory of Darlington (*cf.* '37) and his school explains the different types of division, pre- and post-reduction, of unequal bivalents as being due to different relationships of the unequal segment, the kinetochore which always divides reductionally and the chiasma which may be formed between the kinetochore

Metaphase Configuration

Anaphase Separation

Equational Reductional

Fig. 8. Diagrammatic representation of the two types of chromatid opening (above) and its separation (below) in the heteromorphic short arm pair of chromosome **D** of *Paris hexaphylla*. The circles represent the kinetochores; the equal segments of the chromatids are shown black and the unequal segments (the satellites) blank. In these figures the long arm pair is not shown.

and the place where the difference shows itself. If no chiasma is formed in this equal segment, the first division will be reductional with respect to the difference. If a single chiasma occurs there, the first division will be equational with respect to the difference. The theoretical consequences, on the assumption of chiasmotypy, of the chiasma frequency on the mode of chromatid disjunction was fully discussed by Mather ('35).

In contrast with this view, from the writer's Neo-two-plane theory it follows, as previously stated (Matsuura '37b, p. 174), that "the disjunction of the distal parts of chromosome pairs will always be in the 1:2 ratio for pre- to post-reduction, no matter how chiasmata are formed, and furthermore if chiasmata do not interfere with the mode of disjunction of the kinetochores".

Which view of these is correct must depend upon reliable data from careful observations in which less dependence upon personal

judgment is necessary. In this respect, the data¹⁾ obtained by Dr. Haga from his analytical study of the behavior of the heteromorphic pair (chromosome **D**) in *Paris hexaphylla* seem to meet our requirements. In this particular strain (**DD**⁻), one member of the bivalent in question is deprived of the satellite at its short arm (**D**⁻), while its mate is normally satellited (**D**). Haga distinguished two types of metaphase configurations in respect of this short arm pair as diagrammatically presented in Fig. 8, 'closed' and 'open'. The proportion of these two types was found in a total 300 PMCs to be 202:98 for 'closed' to 'open'. This is very close to the 2:1 ratio.*) At anaphase two types of division are easily distinguishable in this heteromorphic pair, reductional and equational, according to as whether the two chromatids bearing the satellite pass to the same pole, or they separate to the opposite poles respectively. With respect to the frequency of these two types of division, he obtained in a total 1000 PMCs the following results (Table 6):

Table 6. Separation of the heteromorphic arm pair (**DD**⁻) at the first anaphase in the PMC. (Haga's data).

Year	Equational	Reductional	Total	Ratio in %
1935	213	40	253	84.2 : 15.8
1937	611	136	747	81.8 : 18.2
Sum total	824	176	1000	82.4 : 17.6

The chiasmotype theory explains the closed-arm configuration as due to the chiasma which was formed in the equal segment but arrested in terminalization by the unequal region. However this assumption is most improbable, because the equal segment in question is of very short length, less than one major coil length of the chromonema and no interstitial chiasm can be expected there. It fails also to explain how closed and open types of configurations (as well as cross- and parallel associations in the foregoing paragraph) occur in the 2:1 ratio and furthermore why the configuration ratio differs so greatly from the separation ratio. From the separation ratio, it must on the assumption of chiasmotypy be concluded that 82.4% of bivalents were cross-overs and of the closed-arm type of configuration.

On the contrary the present theory explains well the occurrence and frequency of these two types of metaphase configurations.

1) Partially unpublished. Cf. Haga ('37).

*) Note added to proof— Later more extended results obtained by Dr. Haga on the same subject showed that the proportion of the closed to the open configuration was 667:333 in the grand total 1000 PMCs!

Since the unequal segment pair always associates together, it follows that an opening of the equal segment in the equational plane results in the closed-arm configuration and an opening in the reductional plane results in the open-arm one. These should occur in the 2:1 ratio which in actual fact is perfectly in accordance with the observed ratio.

With respect to the question why the separation ratio is so different from the configuration ratio, the present theory seems to fail in its logical interpretation, but it must be noticed that here we are dealing with a specific case to which the present theory is not directly applicable, because the closed-arm type of configuration in which the "chiasma" is obligatorily formed is a type which can never be found in the normal bivalents. In bivalents where no chiasmata are usually formed as in *Trillium kamtschaticum*, the repulsion force between the four arms of a bivalent can reach its equilibrium, resulting thus in the cruciferous type of bivalents. In the closed-arm configuration in question, however, the repulsion force between the two arms is not satisfied owing to the retention of the "chiasma", but is still acting to expand them outwards (in the direction of the arrows in Fig. 8). This is supposed to interfere with the free division of the associated kinetochores and more numerous equational divisions than expected will result from this type of configuration. In the open-arm configuration, however, the division of the kinetochore will be normal, because there is no such force affecting it. (Of course, the chiasma next to the kinetochore in the long arm pair of this bivalent is to interfere with the kinetochore division in the same manner, but this is here conventionally kept out of consideration.) As a consequence, the proportion of the equational division will range from 66.7% to 88.9% according to the minimum and the maximum interferences, respectively, in the closed-arm configuration, as calculated below:

	Closed	Open	Ratio E/R
Configuration ratio (in %)	66.7	33.3	
Separation ratio (in %)			
{ Minimum interference	44.4E : 22.2R	22.2E : 11.1R	66.7E : 33.3R
{ Maximum interference	66.7E : 0R	22.2E : 11.1R	88.9E : 11.1R

In this way of interpretation, the present data are explained by assuming 90.25% interference in the closed-arm type. Since this interference by chiasmata is regarded as a function of the loop length between the kinetochore and the first chiasma, such a high grade interference is expectable from the unusually short length of the chiasma internode in this particular arm pair.

The above interpretation is based on the assumption of the normal pairing of the kinetochore. If conditions prevent their pairing, the first division of the kinetochore will always occur in the reductional plane. This is supposed to be what happens in the majority of cases of unequal sex-chromosome pairs and in the case of such an unequal bivalent as found in *Triticum* by Huskins and Spier ('34) which differs in the position of the kinetochore between the homologues and always segregates reductionally.

In the present view of the interference by chiasmata, it can be seen what rôle the chiasma plays in the chromatid disjunction and how different behavior of bivalents is to be expected among organisms differing in the distribution and nature of the chiasma. Probably in these respects only, the chiasma will be of significance. It may be added here that the results from a series of experiments on the origin of chiasmata¹⁾ which have been carried out in our Laboratories seem to support the writer's present view.

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1) To be published later as a paper of this series.