THE CAUSAL SEQUENCE OF MEIOSIS

II. CONTACT POINTS AND CROSSING-OVER POTENTIAL IN A TRIPLOID *FRITILLARIA*

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(With Four Text-figures)

1. Introduction

Fritillaria latifolia has a giant form, *major*, which was collected by Elwes in the Caucasus. The species is diploid and its variant triploid. The importance of this relationship lies in the special properties of chromosome pairing and crossing-over shown by the diploid in common with most of its nearest relatives. Pairing is interrupted and crossing-over is in consequence confined to the regions near the centromere. If the diploid is so restricted, how will the triploid behave?

In triploids there is the same two-by-two pairing at pachytene as in diploids, so that there is the same limit to the length of chromosome that can be paired. Nevertheless, the total amount of crossing-over per nucleus or per configuration is increased. At least so we find in *Tulipa*, *Hyacinthus*, *Fritillaria lanceolata* and *Drosophila*. It so happens that the amount of crossing-over per chromosome remains about the same. And this might suggest that the chromosome itself is the unit of *crossing-over potential*. Such a simple formula however hides the problem rather than explains it. The different configuration at pachytene and, in *Drosophila*, the different distribution of crossing-over warn us that the similarity of total frequency must be due to the compensating effects of several different intermediate processes.

An obvious difference in these intermediate processes is that pairing in the triploid takes place in a larger nucleus with more chromosomes to sort themselves out. We already know that this leads in tetraploids to a reduced chiasma-frequency as compared with diploids, a change which is measured by Upcott's reduction factor (1939*b*). Each of the three chromosomes of the triploid, on the other hand, has a choice of two partners, which should make the finding of one of them easier. The effect of triploidy on crossing-over should therefore be a balanced one and, from the numerical conditions alone, we could not well predict either an increase or a decrease.

There is yet another intermediate process, however, which might have a positive and predominant effect on the triploid, namely change of

partner. Upcott (1939*a*) has shown that, in *Tulipa* at least, changes of partner are more frequent in triploids than in tetraploids. The odd chromosomes being insaturable, as we may say, seem to provide the most variable sequence of zygotene conditions.

Is the increase in crossing-over of triploids per unit of length paired in fact due to the chromosomes often beginning to pair sooner at a second point? Contact at several points would anchor the chromosome; it would prevent the intercalary parts uncoiling before they have paired and thus releasing the internal torsion, which I assume to be necessary for crossing-over. Two point pairing would reserve the intercalary crossing-over potential; it would increase the frequency of crossing-over and change its distribution.[1]

To test this view a plant with localized chiasmata should be of critical value for distribution as well as frequency can then be compared. Haga (1937*a*, *b*) has described a triploid form of *Paris hexaphylla*, a species with extreme localization of chiasmata near the centromeres. He fails however to take advantage of his material. "The mode of pairing" he concludes "is the same as in diploids, except the number of homologues". His photograph indeed shows that the proximal localization of the diploid is maintained in the triploid. The pairing region evidently remains so short that there are few changes of partner at pachytene, and all the extra chromosomes can appear as univalents at first metaphase, a condition unknown in other autotriploids. Trivalents fail most frequently in the shortest chromosomes.

We have another opportunity in *Fritillaria latifolia*. Frankel (1940) has shown, by classification of the chiasma structure of the diploid and its comparison with other species, that there is usually a single point of contact between the pairing chromosomes, that this point is near the centromere, that pairing is interrupted before it is complete and that pairing itself is slower in the two long *M* chromosomes with median centromeres than in the ten shorter *S*'s with subterminal centromeres. All these are properties characteristic of procentric localization and I shall consequently make use of the conclusions I have reached elsewhere in examining this process in terms of the prime variables of meiosis (1940*b*).

2. General character of the triploid

A first glance at the pollen mother cells of the triploid seems to show little evidence of the procentric localization of the diploid species. This is

[1] I am fortified in this conclusion by learning that Prof. J. B. S. Haldane has arrived at it independently.

especially true if we take cells with the highest number of chiasmata and the fewest univalents (Fig. 1). The trivalents indeed resemble those of the unlocalized *F. lanceolata* both in the distribution of their chiasmata and in the types of their co-orientation (Darlington, 1936). Cells with fewer chiasmata and more univalents begin to show evidences of incomplete, proximally localized, pairing. Comparison of the cells or configurations with different numbers of chiasmata may thus be taken,

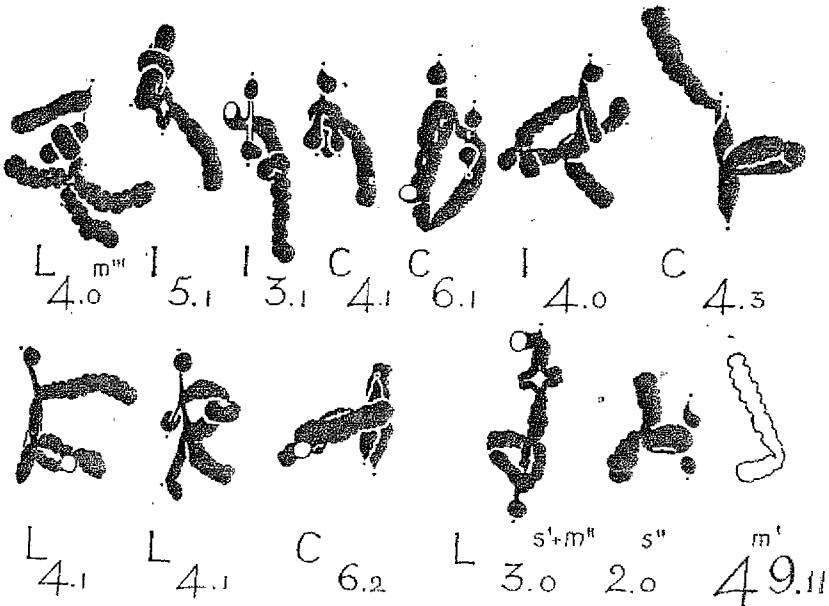


Fig. 1. Complete nucleus of pollen mother-cell of *Fritillaria latifolia major* at first metaphase, with forty-nine chiasmata, eleven terminal (no. 12 in Table II). Numbers of chiasmata under each configuration, co-orientations linear, convergent or indifferent (*L*, *C* or *I*). Eleven trivalents, one being due to illegitimate crossing-over between *M* and *S* chromosomes, not found elsewhere; one *S* bivalent and one *M* univalent. Smear preparation by La Cour, fixed in medium Flemming, stained in gentian violet. ×2000.

as in the diploid, to show stages in the process of pairing, stages at which it can be interrupted. It is on the average interrupted at a stage when far more chiasmata can be formed than in the diploid, yet still not so many as in the triploid of the unlocalized, unrestricted *F. lanceolata*. Hence instead of 0–2 univalents we find 0–8 (Table I).

A record of twelve complete cells, an apparently random sample, gives more precise information. In the first place the chiasma frequency is, as in other triploids, about 50 % greater than that of the corresponding diploid, but retains an equal frequency for *S* and *M* chromosomes

(Table II). Further the proportion of trivalents is slightly higher for the *S* chromosomes (74 %) than for the *M* chromosomes (63 %). The trivalents of both types together have half as many chiasmata again as the bivalents just as in *Tulipa* (Table II). Once more we have an

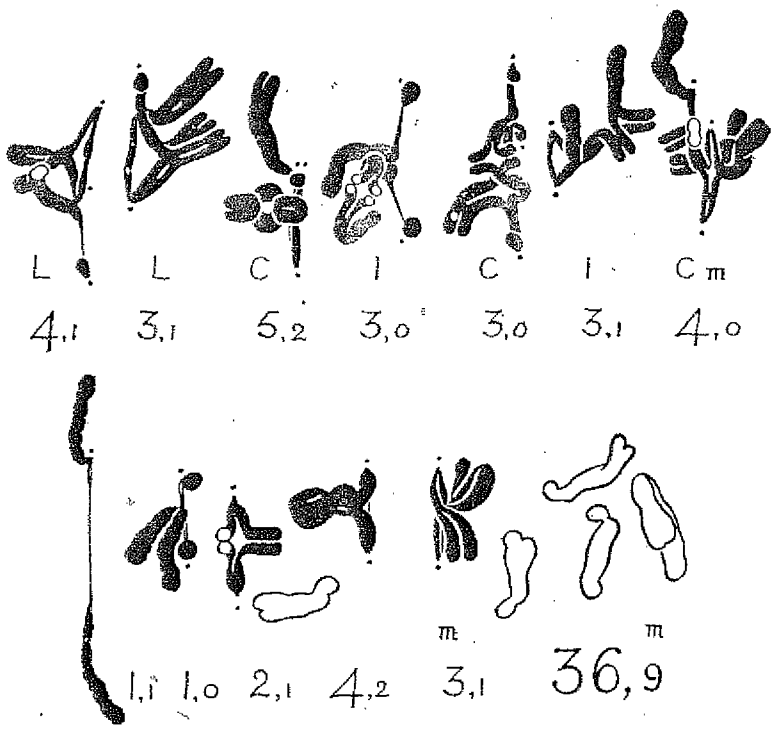


Fig. 2. Nucleus with five bivalents and five univalents (lower row). The chromatid attraction is beginning to lapse at the end of metaphase, but not simultaneously in all parts of the chromosomes (no. 5 in Table II). ×2000.

TABLE I

Frequency of trivalents in thirty-three pollen mother cell nuclei

Trivalents	4	5	6	7	8	9	10	11	12
Nuclei	1	—	2	4	4	7	6	8	1

Mean per nucleus, 9·1.

indication that the extra chromosome is increasing the crossing-over potential in mere proportion. This can be tested by carrying the analysis a step further.

3. Change of partner

The configurations can be classified not merely into bivalents as against trivalents but more exactly into configurations derived from zygotene arrangements with 0, 1, 2, 3, or 4 changes of partner. Of

course, these are minimum changes of partner, that is, those still indicated by associations which have led to the formation of chiasmata. Those with no change of partner produce bivalents and univalents, the rest different kinds of trivalents (Fig. 3).

We then find that the *M* chromosomes never have more than two changes of partner. Their average number is 0·75 as against 1·1 for the *S* chromosomes (Tables III and IV). We can represent this as a difference between 1·75 and 2·1 effective pairing blocks. It agrees in kind with the difference between *M* and *S* types of chromosomes (labelled *L* and *M* respectively) in *Hyacinthus* (Stone & Mather, 1932). Here the pairing

TABLE II

First analysis of chiasmata in twelve nuclei of F. latifolia major

No.	Total chiasmata	No. III ($S+M$)	Chiasmata	
			III	II
1	33	7+1=8	29	4
2	34	6+2=8	25	9
3	35	4+0=4	14	20
4	36	8+1=9	28	8
5 *D*	36	6+1=7	25	11
6	37	9+1=10	31	6
7	37	8+2=10	33	4
8	38	6+1=7	25	13
9	40	8+1=9	32	8
10	42	9+1=10	37	5
11	43	9+2=11	41	2
12 *D*	49	9+2=11	47	2
Total	461	89+15=104	367	94
Mean	38·2	7·4+1·3=8·7	3·5	2·4

blocks were calculated, not directly from the structure of whole configurations, but indirectly from the curve of variation in the numbers of chiasmata in individual chromosomes. Both results mean that the longer *M* chromosomes are slower in movement than the shorter *S*'s, a conclusion we reach equally in *Fritillaria* from the equality of their chiasma frequency.

We can next take the numbers of changes of partner in relation to the chiasma frequencies (Table III). Since $n+1$ chiasmata are necessary to reveal n changes of partner, an absence of causal relationship between change of partner and chiasmata would be shown if the lower chiasma classes were merely cut away in the higher change-of-partner classes. We find this is true of the *M* chromosomes. The mean chiasma frequency of *M*'s is almost the same in all change-of-partner classes. The slight increase in the higher classes agrees with our first simple assumption that lower chiasma classes are cut away in these classes. Change of

partner, in so far as it is still demonstrable at metaphase, has no effect on chiasma frequency in the *M*'s. At the same time the fact that chiasma frequency is 50 % higher than that of diploid *M*'s equally in bivalents and trivalents warns us that conditions are different in the triploid.

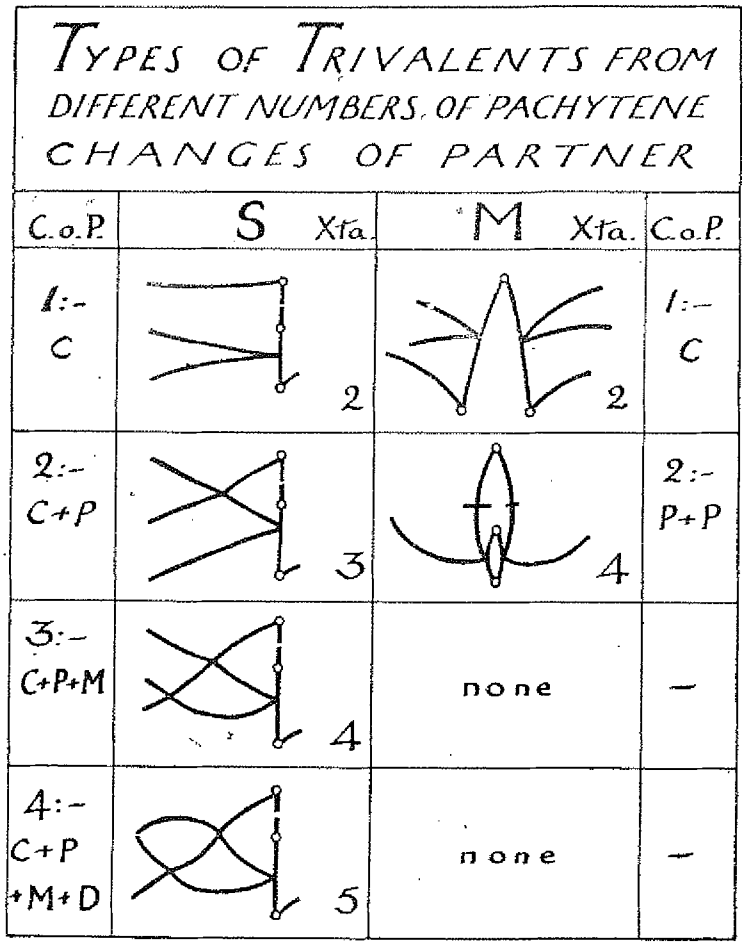


Fig. 3.

Perhaps with a choice of partner in the triploid all pairing is quicker and therefore more effective in storing torsion for crossing over.

The *S* chromosomes tell a different story. The chiasma frequency increases regularly with the number of changes of partner. But this increase is only in part due to lower chiasma classes being cut off in the higher change-of-partner classes, for the mode shifts with the mean. The

apparent effect of change of partner in increasing crossing-over in the *S* trivalents therefore seems to be a true and inherent one. Nor are particular instances to be disregarded where they show the limits of variation. One *S* trivalent with three changes of partner has nine chiasmata, more than twice as many as have been seen in any bivalent of the diploid.

Two conclusions to be drawn from this comparison are of immediate value. First, the most important effect of change of partner on crossing-over occurs with a single change. A second change has less effect. This is easy enough to understand. Anchorage at the two ends has an effect which is not greatly increased by anchorage at a third point.

TABLE III

Relation of chiasma frequency to numbers of changes of partner in S and M configurations (same twelve nuclei)

Change of partner		Xta	1	2	3	4	5	6	9	Total	Xta	Mean
		0	11	8	8	4	—	—	—	31	67	*2·16*
		1	×	9	25	11	5	1	—	51	168	*3·29*
S		2	×	×	15	16	1	1	—	33	120	*3·70*
		3	×	×	×	2	1	—	1	4	22	*5·50*
		4	×	×	×	×	1	—	—	1	5	*5·00*
		0	—	3	3	2	1	—	—	9	28	*3·11*
M		1	×	4	3	4	1	—	—	12	38	*3·17*
		2	×	×	2	1	—	—	—	3	10	*3·33*
S, total		—	*11*	*17*	*48*	*33*	*8*	*2*	*1*	*120*	*382*	*3·18*
M, total		—	—	*7*	*8*	*7*	*2*	—	—	*24*	*76*	*3·17*

Secondly the effect of change of partner is different in *M* and *S* chromosomes. In fact the absence of effect in *M* chromosomes verifies its significance in *S* chromosomes. They act as mutual controls.

For this contrast one or more of the differences between conditions of pairing in *M* and *S* chromosomes might be responsible. Of these we already know that pairing is less complete and chiasmata farther apart in the *M* chromosomes. A second important difference consists in the place where chromosomes change partner. This we must now consider.

4. Order of pairing

Changes of partner and chiasmata may be classified by position as well as frequency. They may occur in any of the arbitrary regions into which Frankel and I have divided the chromosomes (Fig. 4). Median and distal changes of partner of course are confined to trivalents with more numerous chiasmata, for pairing which produces few chiasmata is confined as in the diploid to the proximal regions where it starts.

We then find that of the thirty-one *S* bivalents none have median or distal (*M* or *D*) chiasmata only, and relatively few have these in addition to short-arm and proximal (*S* and *P*) chiasmata. But amongst

forty-eight trivalents with one change of partner one-fifth of the individual chromosomes have median or distal chiasmata only (Table IVA). The distal associations arise independently only when proximal associations are there already. As in the diploid, proximal contact comes first.

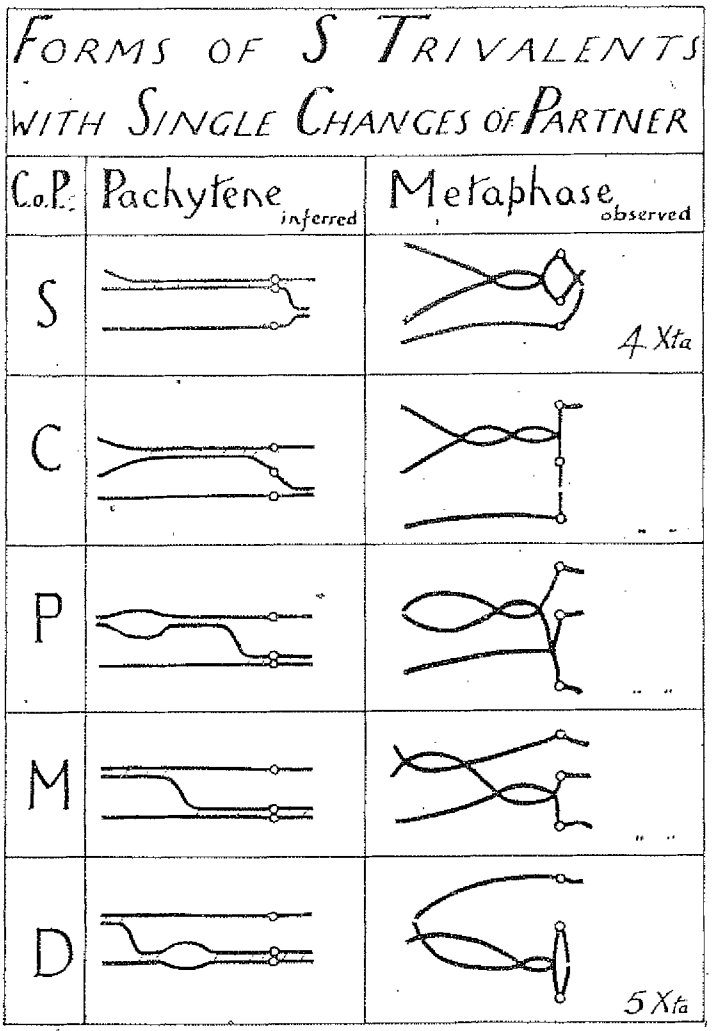


Fig. 4.

In configurations with two changes of partner, individual chromosomes can no longer be identified and we have to consider changes of partner in whole configurations. These show a striking difference, not essentially between configurations with different numbers of changes of

partner, but between those with different numbers of chiasmata. The low chiasma configurations have their changes of partner concentrated in the centric and median regions. The high chiasma configurations have the distribution evened out. This is because the pachytene pairing itself has spread in the high chiasma configurations to the whole length of the chromosomes from the first and second points of contact (Table IVB).

TABLE IV

Segmental distribution of chiasmata (Xta) and changes of partner (C.o.P.) in F. latifolia *3x (twelve nuclei)*

A. Positions of Xta (individual chromosomes)

C.o.P.		No.	(*S*) *P* only	(*S*) *P* and *MD*	*MD* only	Total
S	0	31^{II}	48	14	—	62
	1	48^{III}	64	52	28	144
M	0	9^{II}	10	8	—	18
	1	12^{III}	16	17	3	36

B. Positions of C.o.P. (whole trivalents)

C.o.P.		Xta	*S*	*C*	*P*	*M*	*D*	Total
S	1	2–3	*1*	*13*	*2*	*15*	*2*	33
		4–6	*2*	*4*	*4*	*3*	*1*	14
		Total	3	17	6	18	3	47
	2	3–6	1	14	16	13	12	54
M	1	2–5	—	9	2	1	—	12

When we look at the positions of change of partner in the two types of chromosome we see that those in the *M* chromosomes are much more largely in the centric region. The two points of contact have then been on either side of the centromere. They are close together, so close in fact that their effect in anchoring the intercalary torsion is negligible. This, then, is why change of partner has no effect in increasing the chiasma frequency of the *M* chromosomes.

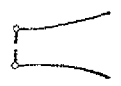
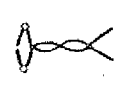
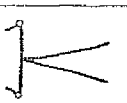
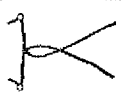
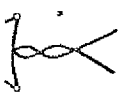
5. BIVALENTS IN THE TRIPLOID

It is not without interest to compare the types of bivalent in the triploid with those found by Frankel in the diploid. Dividing the *S* bivalents into eight classes, four with and four without short arm chiasmata, we see that the distribution of types is in fact different in two ways.

First, the variance in chiasma-frequency is higher in the triploid bivalents. The *M* bivalents in the triploid show increase of variance over those of the diploid and at the same time an increase of mean frequency. There can be no doubt that the triploid bivalents retain the essential properties of procentric localization. But they are a selected sample

(29 % of the whole). The process of selection favours some that have been extremely ill-favoured and others that have been extremely well-favoured for pairing. This is not surprising if we assume that distance apart at leptotene determines the earliness and amount of pairing, since, when all three homologues are very far apart, and when two are very close

TABLE V

F. LATIFOLIA: 5 BIVALENTS IN 2x & 3x						
Xta.		1	2	3	4	T
x	A					
mean	B					
2x	A	2	40	28[t]	7	77
2.33 Xta.	B	9	12	2[t]	–	23
3x	A	2	11	11[t]	6	30
2.24 Xta.	B	15[m]	9[mm]	5[t]	2	31
2x	T	11	52	30	7	100
3x	T	17	20	16	8	61

[t] One bivalent has its distal chiasma nearly terminal.
[m] One bivalent has its proximal chiasma median.
Note. The triploid bivalents in this table and the next include an extra sample beyond those of the twelve cells used in the general analysis. Some discrimination no doubt occurred in selecting these samples but it seems to have reduced the difference between the diploid and the triploid rather than otherwise.

together while the third is far apart, trivalent formation should be equally unlikely. Hence the increase in variance.

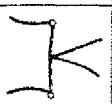
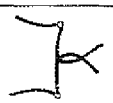
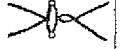
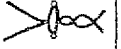
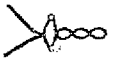
Secondly the frequency of chiasmata in the short arm is lower in the triploid bivalents. A chromosome without a chiasma in the short arm has lost the chance of a change of partner in the centric regions; it has lost 34 % of its chance of forming a trivalent.

6. CONTACT POINTS AND EFFECTIVE TORSION

There remains yet another way of comparing the properties of diploid and triploid. Variance in chiasma frequency per configuration may be taken as evidence of crossing-over mechanism and has been used in this way by Haldane (1931). The variance is low in diploid species of *Fritillaria* with localized chiasmata, with, that is, a single contact point

TABLE VI

F. LATIFOLIA: *M* BIVALENTS IN 2x & 3x

Xra		1	2	3	4	5	T
x No. mean	A			–	–	–	
	B	–				–	
	C	–	–				
2x	A	2	2	–	–	–	4
20^{II}	B	–	11	4	–	–	15
2.20 Xra.	C	–	–	–	1	–	1
3x	A	1	–	–	–	–	1
15^{II}	B	–	5	2	3	–	10
2.6.1 Xra.	C	–	–	1	–	1	2
2x	T	2	13	4	1	–	20
3x	T	1	5	3	3	1	13

in pairing. In unlocalized species, where we have reason to believe there is often a second distal point of contact, this variance is increased out of proportion to the mean. In the triploid it is again greatly increased. And although the *S* chromosomes have the same chiasma-frequency as the *M*, their variance is differentially affected. It is much higher. Again it is in these chromosomes that the average number of changes of partner is so high. And the number of changes of partner is a minimum index of the number of contact points in pairing.

There is thus a *prima facie* case for regarding the establishment of a second contact point in pairing as a means of increasing the frequency of crossing-over disproportionately to the increase in length of chromosome paired.

This principle if true must lie at the root of the mechanics of crossing-over in diploids as well as polyploids. It must also have immediate application to the understanding of various kinds of "asynapsis". But

TABLE VII

Relation of the number of effective pairing blocks to the variance in chiasma frequency per configuration (derived from Tables III, V and VI)

F. latifolia		Chiasma frequency			Mean effective pairing blocks (=changes of partner + 1)
		Mean	Variance	V/M	
2*x*	*M*	2·20	0·48	0·22	*ca.* 1·1
	S	2·33	0·59	0·25	*ca.* 1·1
3*x*	*M*	3·17	0·93	0·30	1·75
	S	3·18	1·81	0·57	2·10

I would not suggest that its proof will be easy or its operation unconditional. If torsion is retained by intermittent contact, the effect of its retention will be greatly affected by the distance of the two contact points apart and by the extent to which the intercalary regions eventually pair. We already see that changes of partner in the *M*'s of our triploid are too close together to have any effect, and we know that a large amount of stored torsion may be wasted in parts of chromosomes which never pair in the diploid *F. Elwesii*.

In order to test this principle we shall therefore need to carry out a similar analysis of other triploids and tetraploids with different types of chromosomes and different conditions of pairing.

SUMMARY

1. *Fritillaria latifolia major* is a triploid form of a diploid species with chiasmata localized near the centromere.

2. The triploid has 3·2 chiasmata per configuration as against 2·3 in the diploid.

3. It has an average of 9·1 trivalents out of 12 possible per cell. The trivalents have an average of 3·5 as against the 2·4 chiasmata of bivalents.

4. *M* chromosomes have the same chiasma frequency as *S* (3·17 against 3·18), whereas in the diploid they have a slightly lower frequency (2·20 as against 2·33).

5. Bivalents in the diploid never have more than four chiasmata and these are always concentrated near the centromere. Configurations in the triploid have as many as nine chiasmata and those with more than four chiasmata show no localization. Those with fewer chiasmata, and especially bivalents, show proximal localization.

6. *M* chromosomes have an average of 0·75 changes of partner shown by chiasma relationships, *S* an average of 1·10, i.e. 1·75 and 2·10 effective pairing blocks. Thus *M*'s move less freely than *S*'s at zygotene and have fewer trivalents at metaphase (63 % against 74 %).

7. The chiasma frequency of *S* chromosomes is higher in configurations with more changes of partner, that is, with more contact points at zygotene (Table III). Its coefficient of variation changes in the same way (Table VII).

8. In *M* chromosomes the second contact point has no effect on the frequency of crossing-over, which is uniformly higher than in the diploid, probably because pairing is made easier by the choice of partner.

9. The difference in effect of change of partner in *M* and *S* is to be correlated with the positions of the points of contact, which are close together in *M*, farther apart in *S*. The effect of two contacts thus depends on the length of segment between them.

10. This effect of two points of contact on the intercalary segment can consist only in their preventing its uncoiling. Such anchorage would preserve the crossing-over potential. The number of contact points is higher in triploids than in diploids, and this difference will therefore account for the increase and change of distribution found in their crossing-over.

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APPENDIX

The chromosomes of the triploid *Tulipa praecox* described by Upcott are similar in shape to the *S* chromosomes of *Fritillaria*. It therefore seems worth while to compare the behaviour of the two in regard to the relationship of changes of partner and chiasma frequency. *T. praecox* has slightly smaller chromosomes with a slightly lower average chiasma frequency (2·90 against 3·18) but about the same average frequency of change of partner (1·15 against 1·10).

Table VIII shows the position in *Tulipa praecox*. The effect of change of partner is similar. The increase of chiasma frequency between the no-change and one-change classes is 0·84 against 1·13 in *Fritillaria*. The tulip is closer to the limits of its crossing-over potential in the no-change class and the effect of a single change of partner is less pronounced. The effect of a second change is negligible. The increase seems to be no more than the inherent selection of the sample requires. Probably beyond a certain frequency per unit of length changes of partner will militate against completeness of pairing at pachytene. That frequency will be lower in *Tulipa* than in *Fritillaria*.

TABLE VIII

Chiasma frequencies of configurations with different numbers of changes of partner in twenty cells of Tulipa praecox ($3x=36$). *Seven configurations include six chromosomes owing to interchange and have four to six changes of partner (after Upcott, 1939a)*

Xta	0	1	2	3	4	5	6	7	8	9	*T*	*M*
0	—	8	21	8	—	—	—	—	—	—	37	*2·00*
1	×	×	40	70	19	—	—	—	—	—	129	*2·84*
2	×	×	×	40	14	1	—	—	—	—	55	*3·30*
3	×	×	×	×	5	—	—	—	—	—	5	*4·00*
4	×	×	×	×	×	—	1	1	1	—	3	*7·00*
5	×	×	×	×	×	×	—	—	1	1	2	*8·50*
6	×	×	×	×	×	×	×	—	—	2	2	*9·00*
T	—	8	61	118	38	1	1	1	2	3	240	2·90