Relation between loss of chromosome associations at metaphase I and interference estimates in rye

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Interference between metaphase I associations (bonds) in specific segments of the chromosomes involved in translocation T242W of rye has been studied. Different values of coincidence were obtained at mid- and late metaphase I for all segment pairs analyzed. At mid-metaphase I there is negative interference between segments opposite to the translocation breakpoint and there is no cross-centromere interference. At late metaphase I there is negative interference in all these segment pairs. The comparison between mid- and late metaphase I cells also indicated that the frequency with which some of these segments are associated decreases along this stage. The possible causes of this decrease and its relation to the differences in coincidence estimates are discussed.

Key words: *Secale cereale* L., translocations, chiasma interference.

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La présente étude porte sur les interférences entre les associations (liaisons) de segments de chromosomes impliqués dans la translocation du T242W du seigle, au cours de la métaphase I. Différentes valeurs de coïncidences ont été obtenues pour toutes les paires de segments analysées à la mi-métaphase I et à la métaphase I tardive. A la mi-métaphase I, une interférence négative survient entre les segments localisés à l'opposé des points de bris de translocation, en ce qu'il n'y a pas d'interférence entre eux et les centromères. A la métaphase I tardive, une interférence négative survient entre toutes les paires de segments. Une comparaison entre les cellules en métaphases I médiane et tardive indique que la fréquence à laquelle certains des segments deviennent associés diminue tout au long du processus métaphasique. Les causes possibles de cette diminution et la relation de celle-ci avec les différences des valeurs de coïncidences sont discutées.

Mots clés : *Secale cereale* L., translocations, interférences chiasmatiques, pertes de bras liés en métaphase I.
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Introduction

In rye, chiasma interference between two chromosome segments belonging to the quadrivalent formed by a translocation heterozygote has been estimated in several instances (Sybenga 1975). In most cases, it was not possible to recognize specific chromosome segments and indirect methods were applied. Additionally, only associations (bonds) between chromosome segments can be observed at meiosis, and interference estimates are based on the assumption that each metaphase I bond is the result of at least one chiasma. However, there are situations in which this last assumption is not realized. Bound arm loss at metaphase I has been described in desynaptic plants of rye (Orellana and Giraldez 1983; Orellana et al. 1984; Cermeño et al. 1984), where it was suggested that the bonds lost were nonchiasmate. This suggestion was based on (*i*) the analysis of the frequency of bound arms at mid- and late metaphase I of plants heterozygous for telomeric C-bands, and (*ii*) the absence of unbound chromosome arms or univalents at late metaphase I that showed evidence of recombination for such C-bands. The existence of metaphase I nonchiasmate bonds in rye has been also concluded from studies that compared the frequency of being bound at metaphase I and the frequency of recombinant chromosomes at anaphase I or metaphase II of specific chromosome arms (Orellana and Giraldez 1983; Orellana et al. 1984; Cermeño et al. 1984; Goicoechea et al. 1987).

In the present work, translocation heterozygotes of rye have been studied at metaphase I. In these heterozygotes the different segments of chromosomes involved in the translocation could be recognized by means of C-banding. A decrease of association frequency from mid- to late metaphase I is reported in such segments, and the effect of this loss in the estimates of chiasma interference is analyzed.

Materials and methods

Meiotic studies were performed in five plants produced by a cross between the following two plants: (*i*) one plant homozygous for translocation T242W from the Wageningen Translocation Tester Set (Sybenga and Wolters 1972), in which chromosomes 2R and 6R are involved (Sybenga et al. 1985); (*ii*) one plant from the selfed offspring of a cross between inbred line E (Giraldez et al. 1979) and cultivar Merced. This plant was heterozygous for an interstitial C-heterochromatin band from cultivar Merced located in the long arm of chromosome 2R.

Three of the plants analyzed were double heterozygotes for translocation T242W and for the interstitial C-band, and the remaining two plants were heterozygotes for translocation T242W and did not have the interstitial C-band. Figure 1 shows the C-banding pattern of the chromosomes involved in translocation T242W, as well as the pairing diagrams of the translocation heterozygotes analyzed.

All plants were grown in a climate chamber under the same conditions. During meiosis, temperature was maintained between 15 and 20°C.

Anthers having PMC at metaphase I and (or) anaphase I were fixed in acetic alcohol 1:3 and maintained in the fixative for 1 to 4 months at 3–4°C. The fixed material was then squashed and stained following the Giemsa C-banding technique described by Giraldez et al. (1979).

Results

In order to take into account the developmental stage of the metaphase I cells studied, anthers having all pollen mother

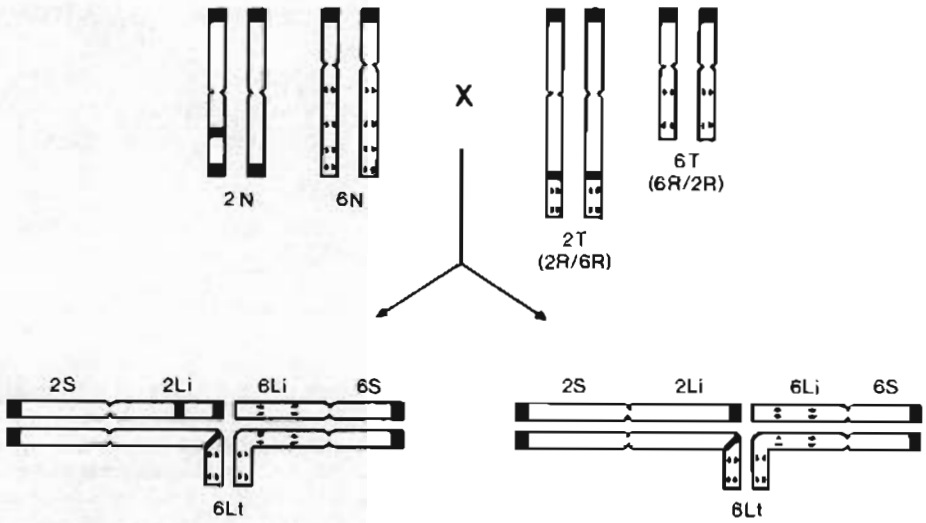

FIG. 1. Diagram of translocation heterozygotes pairing and chromosome constitution of parents. 2S and 6S, unchanged arms; 6Lt, translocated segment; 2Li and 6Li, interstitial segments.

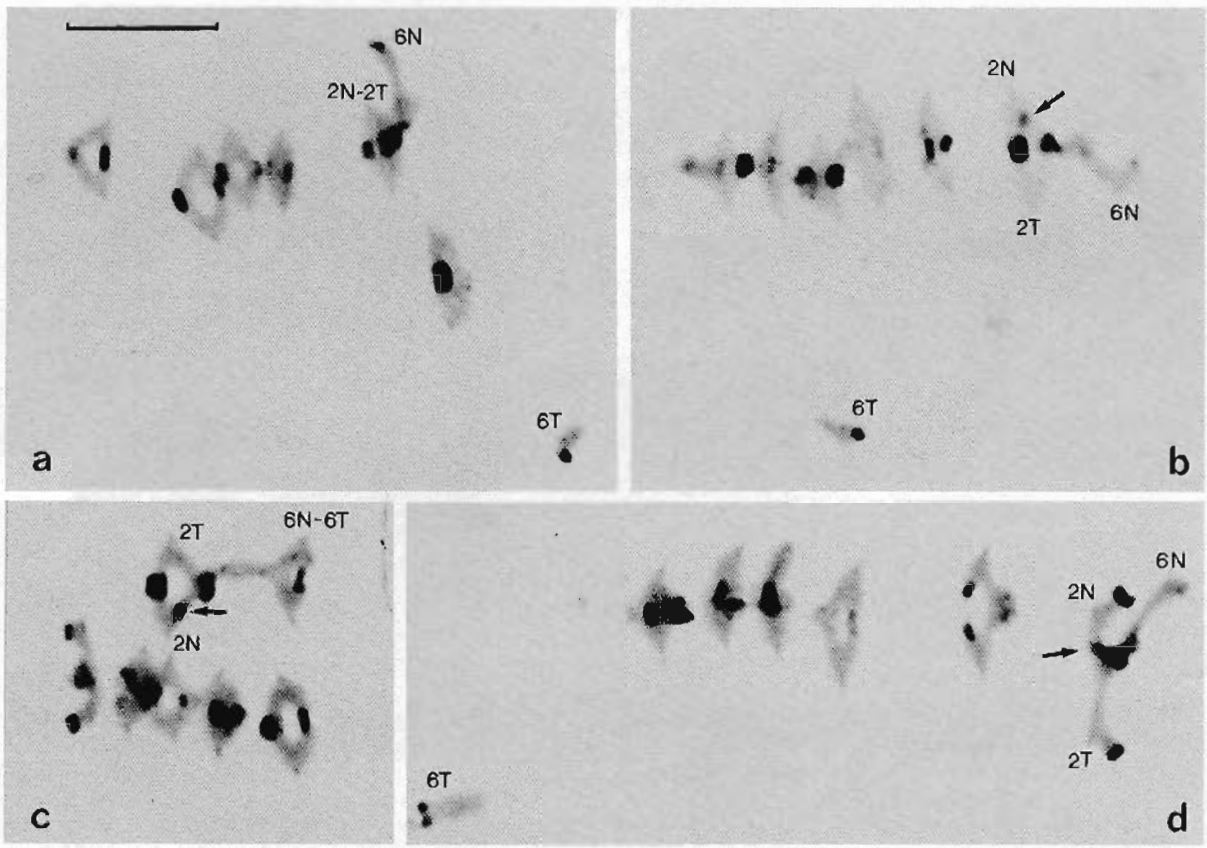

FIG. 2. (*a*) Metaphase I cell of plant without interstitial C-band: trivalent (2S, 2Li, and 6Lt bound) and univalent (translocated chromosome 6T). (*b*–*d*) Metaphase I cells of plants with the interstitial C-band. (*b*) Trivalent and univalent (2S, 2Li, and 6Lt bound). (*c*) Quadrivalent (2S, 2Li, 6Lt, 6Li, and 6S bound). (*d*) Trivalent and univalent (2Li and 6Lt bound). Arrows show the interstitial C-band. Bar, 10 μm.

cells (PMC) at metaphase I (mid-metaphase I) and anthers having PMC simultaneously at metaphase I and anaphase I (late metaphase I) were considered separately.

Using C-banding it was possible to recognize the different segments (Fig. 1) of the four chromosomes involved in the translocation of the two types of plants analyzed (with and without the interstitial C-band): 2S, unchanged short arm of chromosome 2R; 6S, unchanged short arm of chromosome 6R; 6Lt, translocated segment (distal segment of chromosome arm 6RL); 2Li, interstitial segment of chromosome 2R (in practice corresponding to the long arm of chromosome 2R); and 6Li, interstitial segment of chromosome arm 6RL.

The different chromosome pairs not involved in the translocation could also be recognized by their C-banding pattern at

TABLE 1. Comparison between the frequencies of bound and unbound segments at mid- and late metaphase I in the plants having the interstitial C-heterochromatin band

Segment or arm	Mid-metaphase I		Late metaphase I		
	No. bound (%)	No. not bound (%)	No. bound (%)	No. not bound (%)	Contingency χ^2
2S	729	21	1093	57	5.363*
	(97.2)	(2.8)	(95.0)	(5.0)	
2Li	646	286	702	448	0.129ns
	(61.9)	(38.1)	(61.0)	(39.0)	
6Lt	750	—	1148	2	—
	(100)	(0.0)	(99.8)	(0.2)	
6Li	163	587	176	974	12.799**
	(21.7)	(78.3)	(15.3)	(84.7)	
6S	581	169	627	523	103.208**
	(77.5)	(22.5)	(54.5)	(45.5)	
1RS	725	25	1087	63	4.728*
	(96.7)	(3.3)	(94.5)	(5.5)	
1RL	748	2	1141	9	—
	(99.6)	(0.4)	(99.2)	(0.8)	
3R (S and L)	1467	33	2237	63	1.072ns
	(97.8)	(2.2)	(97.3)	(2.7)	
4R (S and L)	1466	34	2225	75	3.221ns
	(97.7)	(2.3)	(96.7)	(3.3)	
5RS	715	35	1096	54	0.001ns
	(95.3)	(4.7)	(95.3)	(4.7)	
5RL	748	2	1144	6	—
	(99.7)	(0.3)	(99.5)	(0.5)	
7R (S and L)	1485	15	2271	29	0.540ns
	(99.0)	(1.0)	(98.7)	(1.3)	

NOTE: ns, not significant; *, $0.05 > p > 0.01$; **, $p < 0.01$.

TABLE 2. Comparison between the frequencies of bound and unbound segments at mid- and late metaphase I in the plants without the interstitial C-heterochromatin band

Segment or arm	Mid-metaphase I		Late metaphase I		
	No. bound (%)	No. not bound (%)	No. bound (%)	No. not bound (%)	Contingency χ^2
2S	1157	43	740	60	15.074**
	(96.4)	(3.6)	(92.5)	(7.5)	
2Li	826	374	530	270	1.467ns
	(68.8)	(31.2)	(66.3)	(33.7)	
6Lt	1199	1	795	5	—
	(99.9)	(0.1)	(99.4)	(0.6)	
6Li	244	956	124	676	7.468**
	(20.3)	(79.7)	(15.5)	(84.5)	
6S	973	227	528	272	58.319**
	(81.1)	(18.9)	(66.0)	(34.0)	
1RS	1039	161	709	91	1.817ns
	(86.6)	(13.4)	(88.6)	(11.4)	
1RL	1194	6	790	10	3.402ns
	(99.5)	(0.5)	(98.7)	(1.3)	
3R (S and L)	2308	92	1516	84	4.580*
	(96.2)	(3.8)	(94.7)	(5.3)	
4R (S and L)	2294	106	1509	91	3.311ns
	(95.6)	(4.4)	(94.3)	(5.7)	
5RS	989	211	651	149	0.353ns
	(82.4)	(17.6)	(81.4)	(18.6)	
5RL	1193	7	797	3	—
	(99.4)	(0.6)	(99.6)	(0.4)	
7R (S and L)	2339	61	1562	38	0.111ns
	(97.5)	(2.5)	(97.6)	(2.4)	

NOTE: ns, not significant; *, $0.05 > p > 0.01$; **, $p < 0.01$.

TABLE 3. Analysis of bond interference between quadrivalent segments 2Li and 6Li (opposite to the translocation breakpoint), 2S and 2Li (across centromere of chromosome 2R), and 6Li and 6S (across centromere of chromosome 6R) at mid- and late metaphase I in plants with and without interstitial C-band

Segments		Plants with the interstitial C-band		Plants without the interstitial C-band	
		Mid-metaphase I	Late metaphase I	Mid-metaphase I	Late metaphase I
2Li	6Li				
B	B	147	140	223	105
B	NB	317	562	603	425
NB	B	16	36	21	19
NB	NB	270	412	353	251
Contingency χ^2		70.787**	29.914**	72.662**	22.286**
Coincidence		C = 1.458	C′ = 1.303	C = 1.328	C′ = 1.278
2S	2Li				
B	B	447	683	794	510
B	NB	282	410	363	230
NB	B	17	19	32	20
NB	NB	4	38	11	40
Contingency χ^2		3.336ns	19.365**	0.648ns	31.433**
Coincidence		C = 0.991	C′ = 1.024	C = 0.997	C′ = 1.040
6Li	6S				
B	B	131	146	196	107
B	NB	32	30	48	17
NB	B	450	481	777	421
NB	NB	137	493	179	255
Contingency χ^2		1.004ns	67.751**	0.114ns	26.923**
Coincidence		C = 1.037	C′ = 1.521	C = 0.991	C′ = 1.307

NOTE: B, segment bound; NB, segment not bound; ns, not significant; **, $p < 0.001$.

metaphase I and, in addition, distinction could be made between the two arms of chromosome pairs 1R and 5R at this stage.

Figure 2 shows examples of metaphase I C-banded cells.

The comparison between the observed association frequency for each chromosome arm or segment (involved or not in the translocation) at mid- and late metaphase I is shown in Table 1 (plants with the interstitial C-band) and Table 2 (plants without the interstitial C-band).

From these results, it can be concluded that there is a significant decrease of associations from mid- to late metaphase I in quadrivalent segments 2S, 6Li, and 6S. Although a general trend towards a shortage of bonds at late metaphase I also appears in the remaining chromosome arms or quadrivalent segments, differences between bound arm frequencies at mid- and late metaphase I were significant in only two cases: chromosome arm 1RS of plants with the interstitial C-band and chromosome 3R of plants without the interstitial C-band.

Table 3 shows the analysis of bond interference, at mid- and late metaphase I, between segments 2Li and 6Li, 2S and 2Li, and 6Li and 6S. Segment 6Lt was not considered in this analysis because it appeared not bound with a very low frequency (see Tables 1 and 2). At mid-metaphase I there is negative interference between segments 2Li and 6Li (opposite to the translocation breakpoint), while there is no interference between segments 2S and 2Li or between 6Li and 6S (across centromeres). At late metaphase I there is negative interference in all cases; i.e., an excess of cells having both segments bound and both segments not bound is found when compared with the expected frequencies under the hypothesis of independence.

Across-centromere coincidence in chromosomes not involved in the translocation could not be estimated due to the low frequency with which chromosome arms were not bound in these bivalents.

Discussion

One possible explanation for the decrease in association frequency between mid- and late metaphase I is that the cells having the lowest association frequency reach anaphase later than the others (Sybenga and de Vries 1987).

Let X be the mean association frequency for a specific segment in anthers having all cells at metaphase I (mid-metaphase I). Let X_1 be the metaphase association frequency in anthers of the same plant where a fraction F of PMC is at anaphase I or later and a fraction $1-F$ is at metaphase I (late metaphase). The cell fraction F comes from metaphase I cells with an association frequency X_2 that, using the above hypothesis, can be estimated from

$$X = (1-F)X_1 + FX_2$$

Table 4 shows the values of F, X, X_1, and X_2 for segment 6S

TABLE 4. The fraction of PMCs later than metaphase I (F), the association frequency for segment 6S at mid (X) and late metaphase I (X_1), and the "expected" association frequency in cells which reached anaphase I or later stages (X_2), in the different anthers analyzed

		Anthers at mid-metaphase I (1 to 6 anthers per plant)	Anthers at late metaphase I				
			1	2	3	4	5
Plant 834	Fraction later than MI (F)	0.00	0.15	0.34	0.49	0.88	
	Association at MI (X or X_1)	0.85	0.76	0.76	0.79	0.51	
	No. of MI cells scored	600	100	100	100	100	
	Expected association frequency of cells later than MI (X_2)	—	1.37	1.03	0.92	0.90	
Plant 835	Fraction later than MI (F)	0.00	0.35	0.36	0.50		
	Association at MI (X or X_1)	0.77	0.82	0.42	0.61		
	No. of MI cells scored	600	100	100	200		
	Expected association frequency of cells later than MI (X_2)	—	0.68	1.39	0.93		
Plant 832	Fraction later than MI (F)	0.00	0.37	0.45	0.62	0.69	0.91
	Association at MI (X or X_1)	0.73	0.68	0.59	0.66	0.32	0.24
	No. of MI cells scored	200	200	210	143	47	50
	Expected association frequency of cells later than MI (X_2)	—	0.82	0.89	0.77	0.92	0.78
Plant 833	Fraction later than MI (F)	0.00	0.57				
	Association at MI (X or X_1)	0.84	0.64				
	No. of MI cells scored	350	100				
	Expected association frequency of cells later than MI (X_2)	—	0.99				
Plant 836	Fraction later than MI (F)	0.00	0.15	0.73			
	Association at MI (X or X_1)	0.71	0.63	0.27			
	No. of MI cells scored	200	200	200			
	Expected association frequency of cells later than MI (X_2)	—	1.16	0.87			

NOTE: Numbers 834 and 835: plants without the interstitial C-band; numbers 832, 833, and 836: plants with the interstitial C-band.

in the different anthers of the plants studied. In 4 out of 15 anthers, the value of X_2 is higher than 1. This might be explained in part by variation in the stage recorded as mid-metaphase and by the large variance of the X_2 estimates. The observation that the average of the X_2 estimates exceeds 1 is a convincing indication that the decrease in association frequency between mid- and late metaphase I cannot be accounted for only in terms of delayed development of cells lacking chiasmata.

The release of associations at late metaphase I (Cermeño et al. 1984) is probably the most important factor in the decrease in association frequency found, although delay may play an additional role.

If the bonds lost were chiasmate, the loss of one chiasma in segment 6Li, even if it was very close to the telomere of chromosome 6T, would only be possible with a simultaneous loss of the chiasmata being formed in region 6Lt. The recombinant chromosome arm thus formed would have been recognized at metaphase I. It could then be concluded that the bonds that were lost in segment 6Li have a nonchiasmate origin, and this conclusion might then be extended to chromosome arms 2S and 6S.

Table 3 indicates that the estimate of coincidence increases from mid- to late metaphase I ($C' > C$) when segment pairs across centromeres are considered (2S and 2Li, or 6Li and 6S), and decreases ($C' < C$) in segments opposite in respect to the translocation breakpoint (2Li and 6Li).

These differences can be explained if the actual value of chiasma interference is obscured by nonchiasmate associations at mid-metaphase I. In this case, $1-C'$ would be a better estimate of chiasma interference than $1-C$. When all nonchiasmate associations have been lost at late metaphase I, $1-C'$ would be a correct estimate of chiasma interference.

An alternative model can be proposed to explain the different values of coincidence as follows. Considering two chromosome segments, S1 and S2, four configurations are possible at early or mid-metaphase I: both segments bound (probability = R); segment S1 bound and segment S2 not bound (probability = A_1); S1 not bound and S2 bound (probability = A_2); and neither S1 nor S2 bound (probability = N). The probability for segment S1 to be bound is $R + A_1$ and the probability for segment S2 to be bound is $R + A_2$. Coincidence C can be estimated as

$$C = \frac{R}{(R + A_1)(R + A_2)}$$

At late metaphase I, after loss of non-chiasmate associations, the frequencies of the four configurations are R', A_1', A_2' and N', respectively, and the new coincidence C' can be estimated as

$$C' = \frac{R'}{(R' + A_1')(R' + A_2')}$$

The relationship between C and C' can be derived as follows. Let the probability for a bond in segment S1 to be lost at late metaphase I be p_1 if segment S2 is bound, and q_1 if segment S2 is not bound, and let the probability for a bond in segment S2 to be lost at late metaphase I be p_2 if segment S1 is bound and q_2 if S1 is not bound, then

$$R' = (1 - p_1)(1 - p_2) R$$

$$A_1' = (1 - q_1) A_1 + p_2 (1 - p_1) R$$

$$A_2' = (1 - q_2) A_2 + p_1 (1 - p_2) R$$

$$N' = N + q_1 A_1 + q_2 A_2 + p_1 p_2 R$$

The estimate of coincidence at late metaphase I will be

$$C' = \frac{R}{\left(R + \frac{1 - q_1}{1 - p_1} A_1\right)\left(R + \frac{1 - q_2}{1 - p_2} A_2\right)}$$

From this equation it can be concluded that

if $p_1 = q_1$ and $p_2 = q_2$, then $C' = C$

if $p_1 > q_1$ and $p_2 > q_2$, then $C' < C$

if $p_1 < q_1$ and $p_2 < q_2$, then $C' > C$

Differences between p_1 and q_1 or p_2 and q_2 could be explained if we assume that the loss of nonchiasmate associations depends on tension forces pulling apart the centromeres (and then the chromosomes) at metaphase I (Nicklas and Kubai 1985). For instance, if we consider the two arms of a bivalent, where one of the arms is bound and the other is not bound (open bivalent), all the tension pulling apart the centromeres is exerted over the bound arm. If both arms are bound (ring bivalent), centromere tension is distributed between the two arms. The probability of a nonchiasmate bond being lost would be higher in the former case. If not all nonchiasmate bonds are lost at late metaphase I, there would be a decrease of open bivalents (because of bound arm loss), which would be relatively higher than that of ring bivalents, resulting in an increase of univalents. As a result, the value of C' would be higher than that of C, independent of the actual value of chiasma interference: an increase of univalents at expense of open bivalents results in negative interference.

Under this hypothesis, when segments opposite to the translocation breakpoint (interstitial segments) are considered, it is not easy to predict the way in which changes of coincidence during metaphase I would be produced since, in this case, two pairs of centromeres are involved in the establishment of the different tensions that would give rise to bond loss.

From the data obtained in a previous report (Goicoechea et al. 1987) in which a comparison was made between the frequency of being bound at metaphase I and the frequency of recombinant chromosomes at anaphase I, it can be concluded that in the plants with the interstitial C-band, segment 6Li has lost all its nonchiasmate associations at late metaphase I. However, in segment 2Li nonchiasmate associations can be still present at this stage. With the present data it cannot be ascertained whether this last possibility can also occur in segments 2S and 6S. The question of whether negative interference found at late metaphase I is a good estimate of the actual chiasma interference is not solved.

The results obtained in this work support the hypothesis of Sybenga (1975), in which negative interference between interstitial segments in translocation heterozygotes is accounted for in terms of pairing difficulties around the translocation breakpoint. Prophase I pairing leads to the appearance of early and mid-metaphase I bound segments that remain associated till anaphase I onset if chiasmata have been formed, or separate (at least in part) during late metaphase I if chiasmata were not present. The negative interference found at mid-metaphase I between interstitial segments 2Li and 6Li can then be considered as the "pairing interference" expected to occur under Sybenga's hypothesis. At mid-metaphase I there is independence between segments 2S and 2Li or 6Li and 6S, agreeing with the expected absence of pairing difficulties around centromeres.

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