Interstitial chiasmata and centromere orientation in heterozygotes for a translocation in rye

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The meiotic behaviour of plants heterozygous for translocation T242W of rye (involving 2RL and 6RL) and an interstitial C-band in 2RL has been analyzed. Chain and frying pan quadrivalents predominate. The following results have further been obtained: (*i*) double chiasmata occur in the interstitial segment carrying the C-band; (*ii*) from the frequency of being bound at metaphase I and the frequency of recombinant chromosomes at anaphase I, estimates of chiasma frequencies (and chiasma interference) in interstitial segments have been derived; (*iii*) estimates of the recombination fraction between the interstitial C-band and the translocation breakpoint have been obtained from offspring analysis; (*iv*) there is a difference in the frequency of alternate orientation between configurations with and without interstitial chiasmata (adjacent-2 has not been observed and a small but significant excess of alternate vs. adjacent-1 coorientation appears). Without intersitial chiasmata, alternate orientation predominates. The possible reasons for these differences are discussed.

Key words: *Secale cereale*, translocations, chiasma frequency, centromere orientation.

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Le comportement méiotique des plantes hétérozygotes concernant la translocation du T242W du seigle, impliquant le 2RL et le 6RL, et une bande C interstitielle chez le 2RL a été analysé. Les quadrivalents disposés en chaine et en poêle à frire ont prédominé. En outre, les résultats suivants ont été obtenus: (*i*) des chiasmas doubles sont survenus dans les segments interstitiels porteurs d'une bande C; (*ii*) des estimés de fréquences de chiasmas (et d'interférences de chiasmas) ont été dérivés à partir de fréquences de chromosomes liés à la métaphase I et de fréquences de chromosomes recombinants à l'anaphase I; (*iii*) des estimés de fractions de recombinaisons entre les bandes C interstitielles et les sites de translocation ont été obtenus par analyse des descendances; (*iv*) une différence s'est présentée dans la fréquence d'alternance d'orientation entre les configurations qui avaient des chiasmas interstitiels et celles qui n'en avaient pas; l'adjacent-2 n'a pas été observé et un excès d'alternance, petit mais significatif, est apparu par opposition à la coorientation de l'adjacent-1. En absence de chiasmas, l'alternance a prédominé. Les raisons possibles de ces différences sont discutées.

Mots clés : *Secale cereale*, translocations, fréquence de chiasmas, orientation des centromères.

[Traduit par la revue]

Introduction

Recently, translocations in rye have been successfully used as cytological markers for chromosomal and gene mapping (de Vries and Sybenga 1984; Figueiras et al. 1985). In this type of structural change, recombination between interstitially located genes and the translocation breakpoint observed in the progeny depends on the chiasma frequency in the corresponding segment and on the orientation (alternate or adjacent) of the multivalents formed (de Vries 1983 and references therein).

In most rye translocations, chiasmata rarely form in interstitial segments, disturbed pairing being adduced as the main reason for this reduced frequency. In the few cases in which recombination in an interstitial segment was demonstrated (de Vries 1983), a maximum of one chiasma was assumed to be formed.

When at least one interstitial chiasma is formed, it is possible to distinguish adjacent-2 orientation from the remaining types (alternate and adjacent-1). In these cases, cytological analyses reveal that adjacent-2 orientation rarely forms, and the results obtained in recombination studies (Kramer and Blander 1961; de Vries 1983) are compatible with a 1:1 ratio of alternate to adjacent-1 orientations. However, up to date, there are no cytological data supporting this last assumption.

The present work deals with the analysis of the meiotic behaviour of heterozygotes for a translocation in rye (*Secale cereale* L.) and with the recombination between the translocation breakpoint and an interstitially located C-heterochromatin band for which the analyzed plants were also heterozygous. Using the procedure described by Giraldez and Orellana (1979), based on the comparison between metaphase I and anaphase I configurations, chiasma frequencies in interstitial segments have been estimated, the possibility of two chiasmata in one of such segments being demonstrated. In addition, alternate and adjacent-1 orientations of multivalents having interstitial chiasmata could be cytologically distinguished.

Materials and methods

Meiotic studies were performed on three plants from a cross between one plant homozygous for translocation T242W of the Wageningen Translocation Tester Set (Sybenga and Wolters 1972), in which chromosomes 2R and 6R (Sybenga et al. 1985) are involved and one plant proceeding from the selfed offspring of a cross between inbred line E (Giraldez et al. 1979) and cv. Merced. This plant was heterozygous for an interstitial C-heterochromatin band located in chromosome 2R proceeding from cv. Merced.

The three plants analyzed were double heterozygous for translocation T242W and for the interstitial C-band.

The offspring obtained from self-pollination of these three plants was mitotically studied for recombination.

To obtain mitotic metaphase cells for karyotype analyses, seeds were germinated on moist filter paper in Petri dishes at room temperature. When the primary roots were 1–2 cm long they were excised and immersed in tap water at 0°C for 48 h to shorten the chromosomes. Subsequently, the tips were fixed in 1:3 acetic acid – alcohol. For

meiotic cells, anthers having PMCs at metaphase I or metaphase I and anaphase I were fixed in 1:3 acetic acid – alcohol.

Both root tips and anthers were 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

The nature of translocation T242W

Figure 1 shows the different metaphase I quadrivalents and trivalents formed by the four chromosomes involved in the translocation. The chromosome segments bound and the coorientation determine which configuration is observed. The pairing diagram shown at the top of Fig. 1 is the basis of these configurations. Figure 2 shows examples of metaphase I C-banded cells.

Translocation T242W is seemingly nonreciprocal because one exchanged segment, if it exists, never contains a chiasma. The points of interchange are located near the middle of the long arm of chromosome 6R and at or near the telomere of the arm of chromosome 2R, which carries the interstitial C-heterochromatin band proceeding from cv. Merced. According to the C-banding pattern proposed for rye in the 1st Workshop on Rye Nomenclature (Sybenga 1983), this would be the long arm of chromosome 2R.

In the four chromosomes involved in the translocation analyzed (2N, nontranslocated 2R chromosome carrying the interstitial C-band; 6N, normal 6R chromosome; 2T, translocated chromosome having a 2R centromere; and 6T, translocated chromosome having a 6R centromere) five segments can be defined: 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. Segment 2Li can be divided into 2Li-1 (between the centromere and the interstitial C-band) and 2Li-2 (between this C-band and the telomere or the translocation breakpoint).

Metaphase I

Using the C-banding technique, it was possible to identify the different segments of the four chromosomes involved in the translocation at meiosis of the plants analyzed. Moreover, segment 2Li-1 (between the band and the centromere) was never bound, which makes it possible to distinguish between alternate (2N 6N – 2T 6T) and adjacent-1 (2N 6T – 2T 6N) orientations in quadrivalents or trivalents having segment 2Li-2 bound and segment 6Li unbound. The interstitial C-band makes it possible to distinguish between the centromeres of chromosomes 2N and 2T (Figs. 1 and 2).

Table 1 indicates the frequencies of the different metaphase I configuration types formed by the four chromosomes involved in the translocation.

From these results the following conclusions can be derived.

Quadrivalents

(*i*) Adjacent-2 orientation (2N 2T – 6N 6T) was never found in 1900 metaphase I cells, (*ii*) All chain quadrivalents found had the four chromosomes in the sequence: 2N, 2T, 6N, 6T (type I chain quadrivalents; Lewis and John 1963). In these quadrivalents a significant excess of alternate coorientations (2N 6N – 2T 6T) versus adjacent-1 ones (2N 6T – 2T 6N) was found. (*iii*) In multivalents having region 2Li-2 bound and region 6Li unbound, a nonsignificant excess of alternate coorientation (2N 6N – 2T 6T) versus adjacent-1 (2N 6T – 2T 6N) was observed.

Trivalents

(*i*) Two types of chain trivalents were found, having the chromosome sequences 2N–2T–6N and 2T–6N–6T. In these trivalents only the "V" orientation was found. (*ii*) In all cases, the two homologous centromeres of the "Y" shaped trivalents oriented to opposite poles.

Bivalents

Two cells were found (0.1%) showing two bivalents and five (0.26%) showing one bivalent formed by chromosomes 2T and 6N and two univalents (2N and 6T). Only in this last case, segregation 2N 2T – 6N 6T can be expected at anaphase I.

Anaphase I

Figure 3 shows the frequencies of the different possible anaphase I cell types with respect to the constitution of the four chromosomes involved in the translocation and their segregation. The minimum number of chiasmata in segments 2Li-1, 2Li-2, and 6Li, necessary for their appearance are also indicated.

Some examples of C-banded anaphase I cells are shown in Fig. 4.

Anaphase I cells showing chromosomes 2N or 6T dividing equationally were found with frequencies 0.011 and 0.095, respectively. The two chromatids of these equationally dividing chromosomes had the same constitution and were then included in the corresponding anaphase I types of Fig. 3 according to the constitution of the remaining chromosomes.

From these results the following can be concluded. (*i*) In agreement with the results obtained at metaphase I, neither 3:1 nor 2N 2T – 6N 6T (adjacent-2) segregations of chromosomes involved in the translocation were found at anaphase I. (*ii*) Recombination in interstitial segments 2Li-2 and 6Li is observed. In addition, 21 cells among 591 studied (3.55%, Fig. 3; Fig. 4*c*) showed evidence of at least two chiasmata in region 2Li-2, involving the four chromatids (comlementary). Only two cells in 591 (0.34%, Fig. 3; Fig. 4*d*) showed evidence of recombination in region 2Li-1 (in one of such cases a second chiasma was formed in region 2Li-2). These results, indicating a very low chiasma frequency in this segment, are consistent with the metaphase I results in which region 2Li-1 was never found to be bound.

Table 2 shows the frequencies of the different gametes expected from anaphase I observations.

Offspring

Four types of chromosome 2R and two types of chromosome 6R appeared in the offspring obtained by self-pollination of the plants studied: parental, translocated 2R/6R without the interstitial C-band (2T); parental, nontranslocated 2R with the interstitial C-band (2N); recombinant, translocated 2R/6R with the interstitial C-band (2Tr); recombinant, nontranslocated 2R without the interstitial C-band (2Nr); nontranslocated 6R (6N); and translocated 6R/2R (6T).

Five types of plants were found in this offspring: normal homozygotes, translocation heterozygotes, translocation homozygotes, duplication heterozygotes (having three copies of segment 6Lt), and duplication homozygotes (having four copies of segment 6Lt). Among the 30 different possible karyotypes, 9 different deficiency heterozygotes or homozygotes can be formed but none were found. The inviability of deficiency

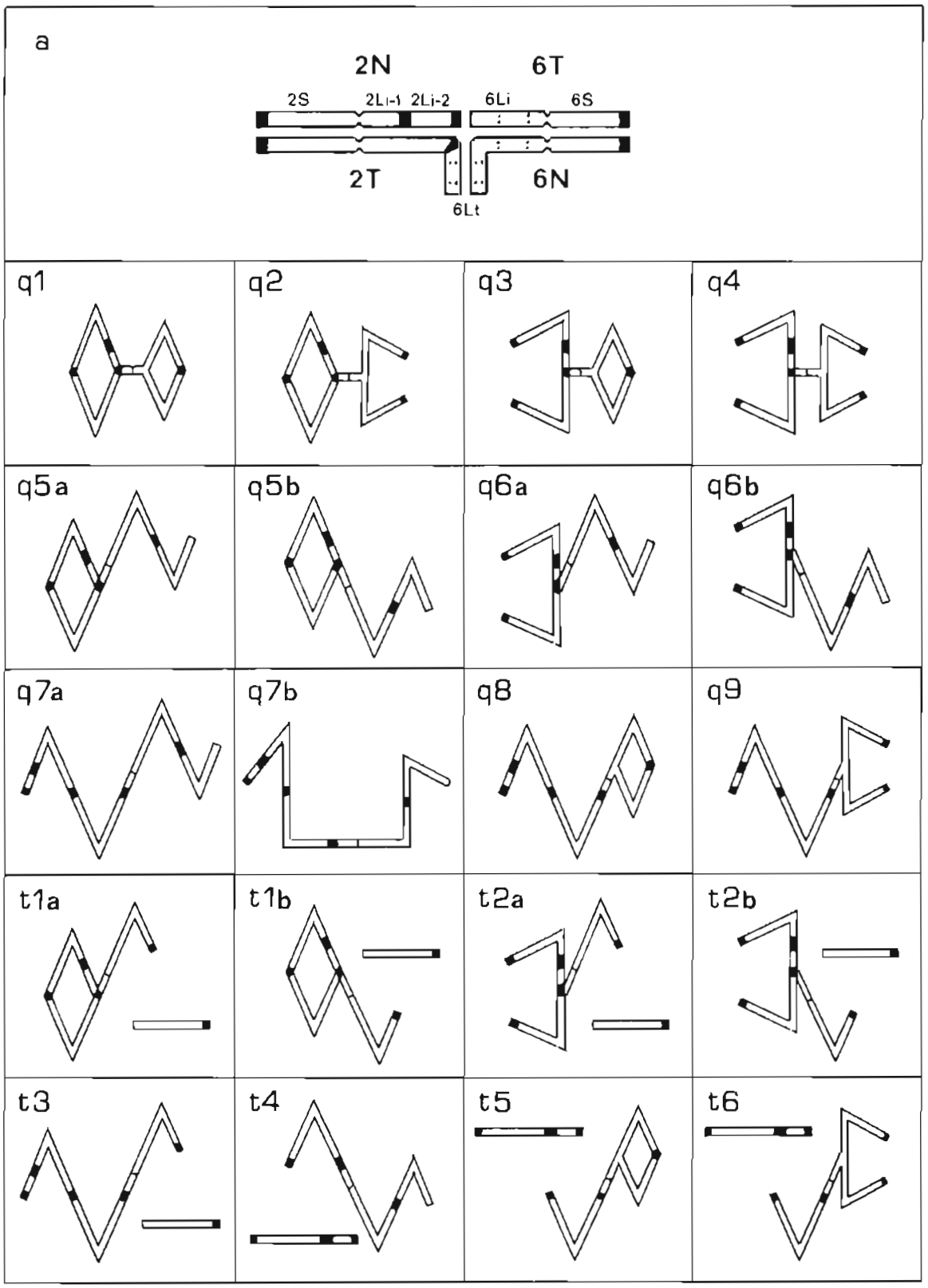

FIG. 1. (*a*) Pairing diagram of the translocation heterozygous plants analyzed: 2S and 6S, unchanged arms; 6Lt, translocated segment; 6Li, interstitial segment of chromosome 6R. Interstitial segment of chromosome 2R is divided into 2Li-1 (between the C-band and the centromere) and 2Li-2 (between the C-band and the translocation breakpoint). q1 to t6 metaphase I configuration types formed by the four chromosomes involved in the translocation according to the chromosome segments bound and the centromere orientation presented (see Table 1).

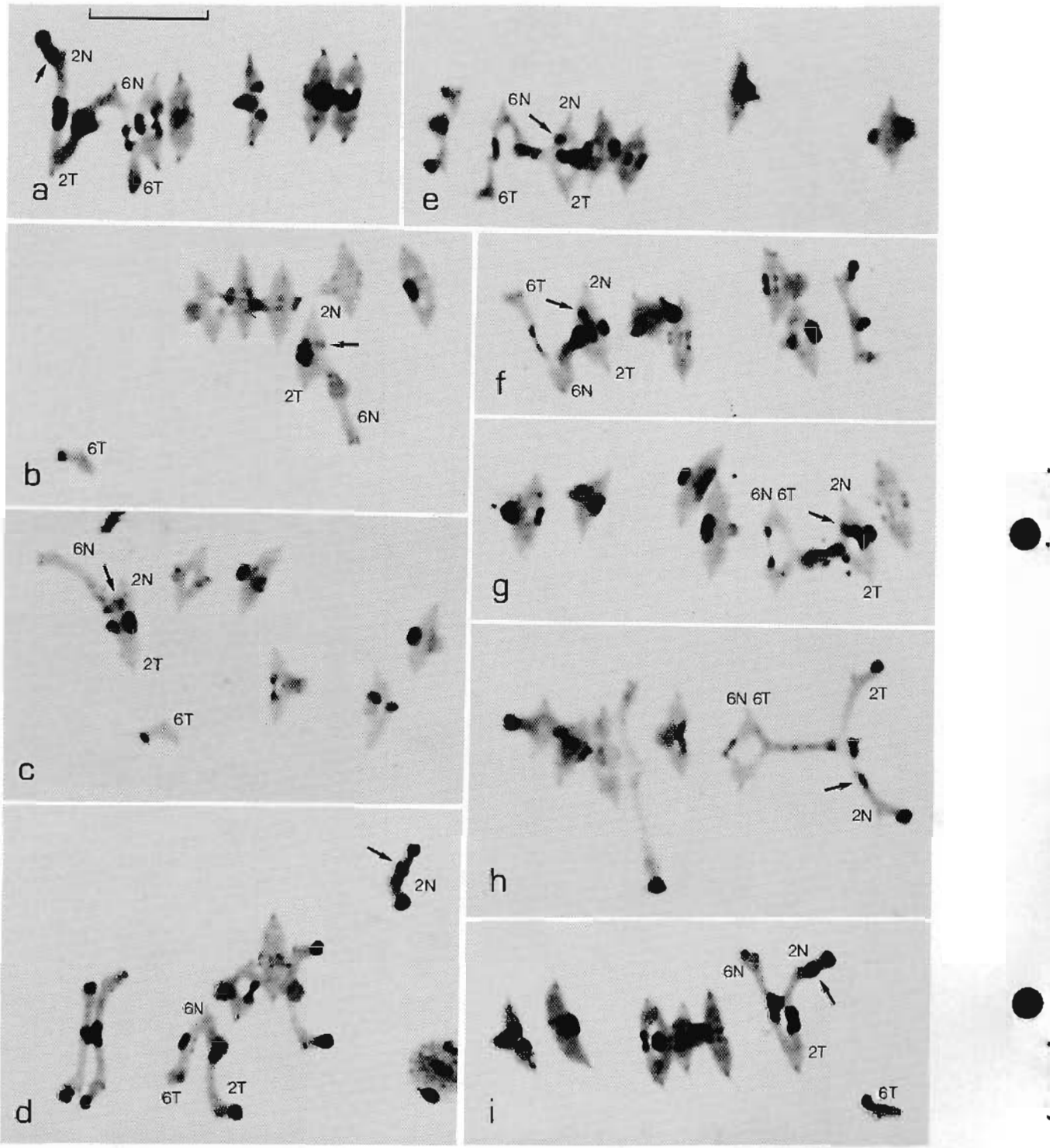

FIG. 2. Metaphase I cells. (*a*) Chain quadrivalent showing alternate orientation (configuration q7a). (*b* and *c*) Trivalents having segments 2S, 2Li-2, and 6Lt bound. (*b*) Adjacent-1 orientation (configuration t1b). (*c*) Alternate orientation (configuration t1a). (*d*). V-shaped chain trivalent involving chromosomes 2T, 6N, and 6T (configuration t4). (*e* and *f*) Quadrivalents having segment 2Li-2 bound and segment 6Li unbound. (*e*) Alternate orientation (configuration q5a). (*f*) Adjacent-1 orientation (configuration q5b. (*g*) Quadrivalent having segments 2S, 2Li-2, 6Lt, 6Li, and 6S bound (configuration q1) with alternate or adjacent-1 orientation. (*h*) Quadrivalent having segments 2S, 2Li-2, 6Lt, and 6Li bound and segment 6S unbound (configuration q2) with alternate or adjacent-1 orientation. (*i*) V-shaped chain trivalent involving chromosomes 2N, 2T, and 6N (configuration t3). Arrow shows the interstitial C-band. Bar = 10μm.

gametes in this translocation was earlier reported by Sybenga and Verhaar (1980).

Since in each plant the four chromosomes involved in translocation could be identified, the chromosome constitution of gametes not forming duplication plants could be deduced. Table 3 shows the frequency of the different normal homozygotes, translocation heterozygotes, and translocation homozygotes found in the offspring, as well as the derived frequency of the different gametes forming such plants.

The constitution of gametes forming duplication plants can be

ANAPHASE I TYPES FOR CHROMOSOMES 6R AND 6R/2R

ANAPHASE I TYPES FOR CHROMOSOMES 2R AND 2R/6R	MINIMUM NUMBER OF CHIASMATA NECESSARY FOR THEIR APPEARANCE	Pole A / Pole B	Pole A / Pole B	Pole A / Pole B
		Segment 6Li	Segment 6Li	Segment 6Li
		0	0	1
Pole A / Pole B	Segment 2Li-1 2Li-2: 0 0	Type I: 238	Type II: 33	Type III: 20
Pole A / Pole B	2Li-1 2Li-2: 0 1	Type IV: 117	Type V: 94	Type VI: 66
Pole A / Pole B	2Li-1 2Li-2: 0 2c	Type VII: 4	Type VIII: 9	Type IX: 8
Pole A / Pole B	2Li-1 2Li-2: 1 0	Type X: 1		Type XI: -
Pole A / Pole B	2Li-1 2Li-2: (1 1)r or (1 1)c	Type XII: -	Type XIII: -	Type XIV: -
Pole A / Pole B	2Li-1 2Li-2: (1 1)d	Type XV: 1	Type XVI: -	Type XVII: -
Pole A / Pole B	2Li-1 2Li-2: 1 2c	Type XVIII: -		Type XIX: -

FIG. 3. The frequency of the different possible anaphase I cell types and the number and positions of chiasmata (see Fig. 1) necessary for their appearance.

TABLE 1. The frequency of the different metaphase I configuration types formed by the four chromosomes involved in translocation. The chromosome segments bound (+) and unbound (−) and the orientation type presented (alternate, 2N 6N – 2T 6T; adjacent-1, 2N 6T – 2T 6N; adjacent-2, 2N 2T – 6N 6T) are also indicated (total number of metaphase I cells = 1900)

Chromosome segments bound					Orientation type (expected segregation)	Corresponding configuration of Fig. 1	Frequency
2S	2Li-2	6Lt	6Li	6S			
					Quadrivalents		
+	+	+	+	+	2N 6N – 2T 6T or 2N 6T – 2T 6N	q1	212
+	+	+	+	−	2N 6N – 2T 6T or 2N 6T – 2T 6N	q2	58
−	+	+	+	+	2N 6N – 2T 6T or 2N 6T – 2T 6N	q3	16
−	+	+	+	−	2N 6N – 2T 6T or 2N 6T – 2T 6N	q4	—
+	+	+	−	+	2N 6N – 2T 6T	q5a	246
+	+	+	−	+	2N 6T – 2T 6N	q5b	222
−	+	+	−	+	2N 6N – 2T 6T	q6a	7
−	+	+	−	+	2N 6T – 2T 6N	q6b	6
+	−	+	−	+	2N 6N – 2T 6T	q7a	376
+	−	+	−	+	2N 6T – 2T 6N	q7b	53
+	−	+	+	+	2N 6N – 2T 6T or 2N 6T – 2T 6N	q8	31
+	−	+	+	−	2N 6N – 2T 6T or 2N 6T – 2T 6N	q9	4
					One trivalent + one univalent		
+	+	+	−	−	2N 6N – 2T 6T	t1a	206
+	+	+	−	−	2N 6T – 2T 6N	t1b	184
−	+	+	−	−	2N 6N – 2T 6T	t2a	5
−	+	+	−	−	2N 6T – 2T 6N	t2b	2
+	−	+	−	−	2N 6N – 2T 6T	t3	228
−	−	+	−	+	2N 6N – 2T 6T	t4	20
−	−	+	+	+	2N 6N – 2T 6T or 2N 6T – 2T 6N	t5	17
−	−	+	+	−	2N 6N – 2T 6T or 2N 6T – 2T 6N	t6	—
					Two bivalents		
+	+	−	+	+	2N 6N – 2T 6T or 2N 6T – 2T 6N	—	1
+	+	−	−	+	2N 6N – 2T 6T or 2N 6T – 2T 6N	—	1
					One bivalent + two univalents		
−	−	+	−	−	2N 6N – 2T 6T or 2N 2T – 6N 6T	—	5

deduced in all cases except in plants having chromosome constitution 2T 2Tr 6T 6N (which can be formed from two different gamete combinations 2T 6T × 2Tr 6N or 2Tr 6T × 2T 6N). Table 4 shows the frequency of the duplication heterozygotes and homozygotes found in the offspring and the derived frequency of the different gametes, with respect to translocation, forming such plants.

An estimate of the recombination fraction (r) between the interstitial C-band and the translocation breakpoint can be made (assuming the same recombination fraction for both female and male gametes) as

$$r = \frac{\text{no. of recombinant chromosomes (2Tr + 2Nr)}}{\text{total no. of chromosomes 2R or 2R/6R}} = 0.274$$

with a standard error of

$$s = \sqrt{r(1-r)n^{-1}} = 0.026$$

The segregations obtained for single factors (translocation segment and interstitial C-band), within the plants not carrying the duplication, were not significantly different from the expected 1:1 ratio.

The comparisons of the frequencies of the different gametes expected from anaphase I observations (Table 2) and the offspring data are also shown in Tables 3 and 4. The differences are not significant.

Discussion

Estimates of chiasma frequencies in interstitial segments

Segment 2Li-2

Considering the evidence of recombination in segment 2Li-2 (and disregarding the extremely low frequency with which chiasmata in region 2Li-1 were formed), three types of anaphase I cells were found for chromosomes 2N and 2T (Fig. 3).

(*i*) Parental chromosomes showing no evidence of recombination. If $f_0, f_1, f_2, \ldots, f_n$ are the frequencies of 0, 1, 2, ..., n chiasmata, respectively, in segment 2Li-2, and assuming no chromatid interference, its expected frequency would be (Mather 1935)

$$[1] \quad F_p = f_0 + 1/4f_2 + 1/8f_3 + \ldots + 1/6(1 - (-1/2)^{n-1})f_n$$

(*ii*) Chromosomes showing evidence of recombination. Its expected frequency assuming no chromatid interference would be

$$[2] \quad F_r = f_1 + 1/2f_2 + 3/4f_3 + \ldots + 2/3(1 - (-1/2)^n)f_n$$

(*iii*) Chromosomes showing evidence of at least two chiasmata being formed. The expected frequency of this anaphase I

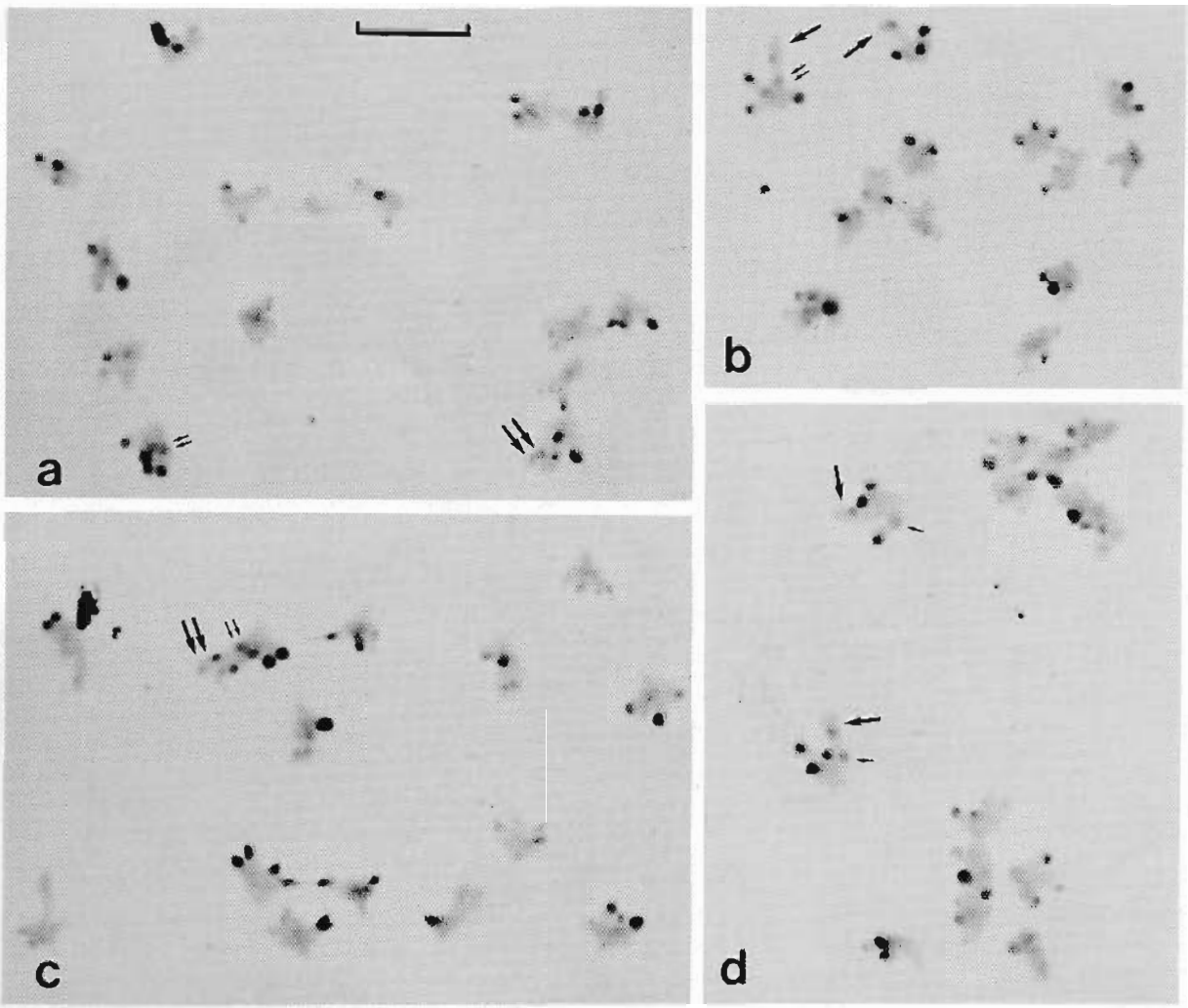

FIG. 4. Anaphase I cells of plants having the interstitial C-band (*a*) Type I cell of Fig. 3. (*b*) Type V cell of Fig. 3. (*c*) Type IX cell of Fig. 3. (*d*) Type XV cell of Fig. 3. Small arrows show the interstitial C-band; big arrows show the translocated segment 6Lt. Bar = 10 μm.

TABLE 2. The frequencies of the different gametes expected from anaphase I observations (Fig. 3). Anaphase I types resulting from at least one chiasmata in region 2Li-1 have not been considered

Anaphase I cell types (Fig. 3)	Contribution to the different types of gametes							
	2N 6N	2T 6T	2Nr 6N	2Tr 6T	2N 6T	2T 6N	2Nr 6T	2Tr 6N
I	1/2	1/2	—	—	—	—	—	—
II	—	—	—	—	1/2	1/2	—	—
III	1/4	1/4	—	—	1/4	1/4	—	—
IV	1/4	1/4	—	—	—	—	1/4	1/4
V	—	—	1/4	1/4	1/4	1/4	—	—
VI	1/8	1/8	1/8	1/8	1/8	1/8	1/8	1/8
VII	—	—	—	—	—	—	1/2	1/2
VIII	—	—	1/2	1/2	—	—	—	—
IX	—	—	1/4	1/4	—	—	1/4	1/4
Frequency of gametes expected from anaphase I observations (Fig. 3)	161.50	161.50	38.25	38.25	53.25	53.25	41.50	41.50

NOTE: 2N, parental nontranslocated chromosome 2R with the interstitial C-band; 2T, parental translocated chromosome 2R/6R without the interstitial C-band; 2Nr, recombinant nontranslocated chromosome 2R without the interstitial C-band; 2Tr, recombinant translocated chromosome 2R/6R with the interstitial C-band; 6N, nontranslocated chromosome 6R; 6T, translocated chromosome 6R/2R.

TABLE 3. Chromosome constitution of normal homozygotes, translocation heterozygotes, and translocation homozygotes from the offspring obtained by self-pollination of the plants studied

Plant chromosome constitution	Frequency	Derived frequency of gametes			
		2N 6N	2T 6T	2Nr 6N	2Tr 6T
Normal homozygotes					
2N 2N 6N 6N	12	24	—	—	—
2N 2Nr 6N 6N	12	12	—	12	—
2Nr 2Nr 6N 6N	2	—	—	4	—
Translocation heterozygotes					
2N 2T 6N 6T	23	23	23	—	—
2N 2Tr 6N 6T	11	11	—	—	11
2Nr 2T 6N 6T	6	—	6	6	—
2Nr 2Tr 6N 6T	3	—	—	3	3
Translocation homozygotes					
2T 2T 6T 6T	14	—	28	—	—
2T 2Tr 6T 6T	9	—	9	—	9
2Tr 2Tr 6T 6T	2	—	—	—	4
Total	94	70	66	25	27
Frequency of gametes expected from anaphase I (Table 2)		161.50	161.50	38.25	38.25
Contingency $\chi^2 = 5.548$ df = 3; $0.2 > p > 0.1$)					

NOTE: See Table 2 footnote for explanation of gamete types. The comparison of the frequencies of gametes derived from these plants and the corresponding frequencies of the different nonduplication gametes derived from anaphase I observations (Table 2) is also shown.

type in absence of chromatid interference would be

[3] $F_d = 1/4f_2 + 1/8f_3 + \ldots + 1/6(1 - (-1/2)^{n-1})f_n$

If the chiasma frequencies $f_0, f_1, f_2, \ldots, f_n$ follow a Poisson distribution (assuming no chiasma interference), the value of the distribution mean (m) can be derived from

$$\text{unbound segments at metaphase I} = f_0 = e^{-m}$$

and subsequently the expected values of $f_1, f_2, f_3, \ldots, f_n$.

The comparison of the expected values of F_p, F_r, and F_d derived from [1], [2], and [3] and the observed ones (Table 5) revealed a significant excess of observed parental anaphase I cells (F_p) and a deficit of the two remaining anaphase I cell types (F_r and F_d).

If a maximum of two chiasmata is considered, then

$$F_p = f_0 + 1/4f_2$$
$$Fr = f_1 + 1/2f_2 = 1 - f_0 - 1/2f_2$$
$$F_d = 1/4f_2$$

Taking f_0 as the number of unbound arms at metaphase I, an estimate of f_2 ($f_1 = 1 - f_0 - f_2$) can be made by maximization of

$$\frac{(F_p + F_r + F_d)!}{F_p!\,F_r!\,F_d!}(f_0 + 1/4f_2)^{Fp}(1 - f_0 - 1/2f_2)^{Fr}(1/4f_2)^{Fd}$$

The values obtained by this maximum likelihood method and the corresponding expected values of F_p, F_r, and F_d are also shown in Table 5. Again, the comparison between these values and the observed ones revealed an excess of parentals (F_p) and a deficit of the two remaining classes (F_r and F_d).

Figure 5 shows the expected values of F_r under the hypothesis of one chiasma per bond (interference = 1) and under the hypothesis of Poisson distribution of chiasma frequencies (interference = 0), plotted against f_0 (estimated as not bound segments at metaphase I). According to this figure, the values of F_r and f_0 would reveal the existence of negative interference (coincidence > 1) in segment 2Li-2. However, if negative chiasma interference was operating in this segment, and excess of recombinant chromosomes formed by at least two chiasmata (F_d) would also be expected. The defect shown by double-recombinant chromosomes (F_d) together with the excess of parental chromosomes (F_p) could be explained in two other different ways.

(*i*) Chromatid interference. Let f_i be the frequency of cells in which more than one chiasma is formed in segment 2Li-2. Then, [1], [2], and [3] can be rewritten as

$$F_p = f_0 + xf_i$$
$$F_r = f_1 + yf_i$$
$$F_d = zf_i$$

in which x, y, and z are the relative contributions of these cells to the corresponding anaphase I types (if a maximum of two chiasmata were formed, x = reciprocal doubles, y = diagonal doubles, and z = complementary doubles). The excess of parental anaphase I chromosomes could be accounted for if $x > z$:

$$F_p = f_0 + (x/z)F_d$$

As shown in Table 5, $F_p = 291/589 = 0.494$; $f_0 = 0.386$; and $F_d = 21/589 = 0.036$. Then,

$$x/z = 3.02$$

i.e., three times more "reciprocal double chiasmata" than "complementary double chiasmata" were formed (it is worth mentioning that this estimate is extremely inaccurate and therefore, can be somewhat misleading).

This would represent negative chromatid interference, a situation that probably has never been studied in plants. The

TABLE 4. Chromosome constitution of duplication heterozygotes and duplication homozygotes from the offspring obtained by self-pollination of the plants studied.

Plant chromosome constitution	Frequency	Derived frequency of gametes: 2N 6N or 2Nr 6N	2T 6T or 2Tr 6T	2T 6N or 2Tr 6N
Duplication heterozygotes				
2N 2T 6N 6N	12	12	—	12
2N 2Tr 6N 6N	2	2	—	2
2Nr 2T 6N 6N	5	5	—	5
2Nr 2Tr 6N 6N	1	1	—	1
2T 2T 6N 6T	15	—	15	15
2T 2Tr 6T 6N	14	—	14	14
2Tr 2Tr 6N 6T	2	—	2	2
Duplication homozygotes				
2T 2T 6N 6N	—	—	—	—
2T 2Tr 6N 6N	1	—	—	2
2Tr 2Tr 6N 6N	—	—	—	—
Total	52	20	31	53
Frequency of gametes from non-duplication plants (Table 3)		95	93	—
Total frequency of gametes derived from offspring		115	124	53
Frequency of gametes expected from anaphase I observations (Table 2)		199.75	199.75	94.75
Contingency $\chi^2 = 0.338$ (df = 2; $0.9 > p > 0.8$)				

NOTE: See Table 2 footnote for explanation of gamete types. The comparison of the total frequencies of gametes derived from the offspring and the frequencies of the corresponding gametes expected from anaphase I observations (Table 2) is also shown.

TABLE 5. Comparison of the observed frequencies of parental (F_p), recombinant (F_r), and double recombinant (F_d) anaphase I cell types for segments 2Li-2 and 6Li, and the corresponding expected frequencies under the following assumptions: *a*, Poisson distribution of chiasmata; *b*, a maximum of two chiasmata being formed

Segment	Assumption	Expected chiasma frequencies: f_0	f_1	f_2	f_3 or more	Frequencies of anaphase I cell types: Expected F_p	F_r	F_d	Observed F_p	F_r	F_d	χ^2
2Li-2	*a*	0.386	0.367	0.175	0.072	258.9	298.5	31.6	291	277	21	9.0621*
2Li-2	*b*	0.386	0.411	0.203	—	257.2	301.9	29.9	291	277	21	9.1447*
6Li	*a*	0.822	0.161	0.016	0.001	488.9	100.1		495	94		0.4376ns

*$0.05 > p > 0.01$.

excess of complementary plus reciprocal versus disparate pairs of chiasmata observed at early diplotene in grasshoppers (for references see Sybenga 1975) would indicate that chromatids are not free to participate at random in two neighboring chiasmata. Negative chromatid interference may, therefore, occur in special systems and then cannot be completely excluded.

(*ii*) Nonchiasmate bonds. The existence of nonchiasmate metaphase I bonds in rye has been demonstrated in several instances (Orellana and Giraldez 1983, 1984; Orellana et al. 1984; Cermeño et al. 1984).

Under this hypothesis, segment 2Li-2 would have three different possibilities at metaphase I: it can be bound having at least one chiasma (with a probability CB = $f_1 + f_2 + \ldots + f_n$), it can be bound having no chiasmata (with a probability NCB), or it can be unbound (with a probability NB). In this case, the relationships between anaphase I and metaphase I results (in absence of chromatid interference) would be

$$F_p = \text{NB} + \text{NCB} + 1/4f_2 + 1/8f_3 + \ldots + 1/6(1 - (-1/2)^{n-1})f_n$$

$$F_r = f_1 + 1/2f_2 + 3/4f_3 + \ldots + 2/3(1 - (-1/2)^n)f_n$$

$$F_d = 1/4f_2 + 1/8f_3 + \ldots + 1/6(1 - (-1/2)^{n-1})f_n$$

Cytologically, only two possibilities can be distinguished at metaphase I: bound segments (B = CB + NCB) and not bound segments (NB). As f_0 = NCB + NB, an underestimation of f_0 would result if f_0 is taken as the number of not bound segments (NB) at metaphase I, and subsequently, an underestimation of expected parental anaphase I chromosomes appears.

If a maximum of two chiasmata is considered, in absence of

chromatid interference, f_0, f_1, and f_2 can be estimated from anaphase I data as

$$f_2 = 4F_d = 0.143$$

$$f_1 = F_r - 1/2f_2 = 0.399$$

$$f_0 = 1 - f_1 - f_2 = 0.458$$

and the frequency of nonchiasmate bonds would be

$$NCB = F_p - NB - 1/4f_2 = 0.072$$

Under this assumption, an estimate of chiasma interference (I) within this segment can be attempted. Coincidence (C) can be estimated from the ratio between "observed" double crossovers (frequency of two chiasmata) and expected doubles (or triples etc.) under the hypothesis of independence (Poisson distribution of chiasmata having a first term, $f_0 = 0.458$). Interference equals 1-C.

$$C = 0.143/0.184 = 0.775;\ I = 0.225$$

The genetic length (L) of segment 2Li-2 would be

$$L = 50\,(f_1 + 2f_2) = 34.25\ \text{cM}$$

Segment 6Li

In segment 6Li (interstitial segment of chromosomes 6N and 6T) there is no cytological evidence of two chiasmata being formed. Then, the possibility that a bond at metaphase I in this segment is the result of only one chiasma can be considered. In this case, the probability for this segment to be bound at metaphase I would equal the frequency of the corresponding recombinant anaphase I cells.

There is no significant difference (contingency $\chi^2 = 1.1092$) between the frequency of the two possible configurations at metaphase I for segment 6Li (bound and unbound) and the frequency of recombinant (F_r) and parental (F_p) anaphase I types.

The possibility of more than one chiasma being formed (assuming no chiasma interference) can also be considered for this segment. As indicated above, the frequencies of 0 (f_0), 1 (f_1), 2 (f_2), ..., n (f_n) chiasmata can be estimated since they would follow a Poisson distribution having a mean (m) that can be derived from

$$f_0 = e^{-m}$$

(f_0 being the frequency with which the corresponding interstitial segment is unbound at metaphase I).

Now, the expected values for F_p and F_r can be derived from Mather (1935)

$$F_p = f_0 + 1/2f_2 + 1/4f_3 + \ldots + 1/3(1 - (-1/2)^{n-1})\,f_n$$

$$F_r = f_1 + 1/2f_2 + 3/4f_3 + \ldots + 2/3(1 - (-1/2)^n)\,f_n$$

and compared with the observed ones. This comparison is also shown in Table 5. There is a good fit between observed and expected data.

Obviously, the expected frequencies of recombinant anaphase I type (F_r) under the assumption of no chiasma interference ($I = 0$) and under the assumption of one chiasmata per bond (interference = 1) approach 0 with increasing values of f_0 (Fig. 5). This is the reason for the fit between the observed values of F_r and F_p of chromosome pair 6R and the expected ones under the two hypotheses.

In segment 6Li there is also an excess of parental anaphase I chromosomes (under the two hypotheses), which, owing to the low frequency of metaphase I bonds, is not significant. This suggests that, also in this segment, nonchiasmate bonds can be present with a low frequency.

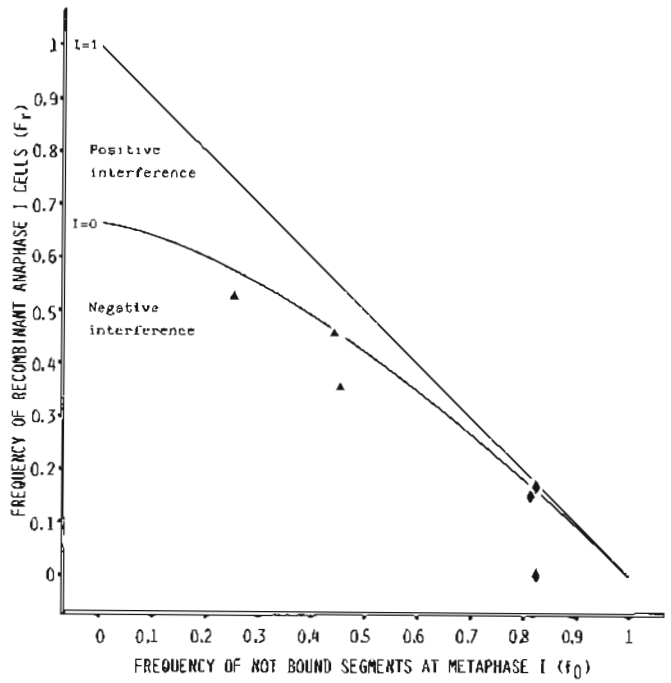

FIG. 5. The frequency of unbound segments at metaphase I (f_0) plotted against the frequency of the corresponding anaphase I recombinant cells (F_r) in the different plants analyzed for segment 2Li-2 (▲) and segment 6Li (◆). The expected values under the hypothesis of one chiasma per bond ($I = 1$) and Poisson distribution of chiasmata ($I = 0$) are also indicated.

Assuming a maximum of one chiasma being formed, the frequencies of 0 (f_0) and 1 (f_1) chiasma in segment 6Li, and subsequently their genetic length (L), can be estimated considering together metaphase I and anaphase I data:

$$f_0 = 0.826;\ f_1 = 0.174;\ L = 8.7\ \text{cM}$$

Multivalent orientation

The results obtained in this work concerning chain multivalent orientation at metaphase I (Table 2) agree with the general findings of an excess of alternate versus adjacent-1 orientations and an absence of adjacent-2 ones in type I chain quadrivalents (i.e., having nonhomologous centromeres in distal positions) and the almost exclusive appearance of "V"-shaped orientations in chain trivalents (for review see Rickards 1983). The absence of anaphase I cells showing homologous centromeres migrating to the same pole (2N 2T – 6N 6T) confirms the absence of adjacent-2 orientations in this material.

Concerning the orientations of multivalents having segment 2Li-2 bound and segment 6Li unbound, a nonsignificant excess of alternate coorientations (2N 6N – 2T 6T; configurations q5a, q6a, t1a, and t2a in Fig. 1) versus adjacent-1 ones (2N 6T – 2T 6N; configurations q5b, q6b, t1b, and t2b in Fig. 1) appears ($\chi^2_{1:1} = 2.847$; $0.1 > p > 0.05$).

Anaphase I cell types showing evidence of recombination in chromosome segment 2Li-2 and not in chromosome segment 6Li proceed from metaphase I cells having segment 2Li-2 bound and segment 6Li unbound. "Alternate" (cell types IV and VII in Fig. 3) and "adjacent-1" (cell types V and VIII in Fig. 3) centromere segregation can be distinguished in such anaphase I cells. Again, a nonsignificant excess of alternates ($\chi^2_{1:1} =$

1.446; $0.3 > p > 0.2$) was observed. Now, if the frequencies of alternate and adjacent-1 orientations at metaphase I and anaphase I are added, a significant excess of alternates appears ($\chi^2_{(1:1)} = 4.196$; $0.05 > p > 0.01$).

The necessity of a physical tension for stability of centromere coorientation has been demonstrated by Bauer et al. (1961), Nicklas and Koch (1969), and Henderson and Koch (1970). Since these tensions can only be established between centromeres belonging to chromosomes physically connected, one of the factors that affect the stabilization of a particular multivalent coorientation (and then the frequency with which it appears) is multivalent shape, which, in turn, is determined by the number and location of chiasmata. The differences in the frequency of alternate orientation between chain and interstitially bound multivalents can be accounted for in these terms. However, other factors different from multivalent shape must be responsible for the slight excess of alternate orientations found within interstitially bound multivalents.

A possibility that can be taken into account is the existence of between-centromeres differences in activity or in the time necessary for reaching activity. The existence of a sequential centromere activation in multivalents has been suggested by Narasinga Rao and Sybenga (1984). In multivalents having segment 2Li-2 bound and segment 6Li unbound, tensions are established between the centromeres of chromosomes 2N and 2T and, in addition, between one of these centromeres and the centromere of chromosome 6N. If, for instance, centromeres of chromosomes 2T and 6N are "more active" than the centromere of chromosome 2N, centromeres of chromosomes 2T and 6N would reach a stable tension with a higher probability than centromeres of 2N and 6N, and a slight excess of alternate orientations would be expected.

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BAUER, H., DIETZ, R., and RÖBBELEN, C. 1961. Die Spermatocytenteilungen der Tipuliden III. Das Bewegungsverhalten der chromosomen in Translokationheterozygoten von Tipula oleracea. Chromosoma, **12**: 116–189.

CERMEÑO, M. C., ORELLANA, J., and LACADENA, J. R. 1984. Evidence of nonchiasmate bonds at metaphase I in inbred rye. Can. J. Genet. Cytol. **26**: 409–414.

DE VRIES, J. N. 1983. High recombination between the breakpoint of a reciprocal translocation in rye (*Secale cereale* L.) and an interstitially located gene. Theor. Appl. Genet. **66**: 329–339.

DE VRIES, J. N., and SYBENGA, J. 1984. Chromosomal location of 17 monogenically inherited morphological markers in rye (*Secale cereale* L.) using the translocation tester set. Z. Pflanzenzuecht. **92**: 117–139.

FIGUEIRAS, A. M., GOZÁLEZ JAÉN, M. T., SALINAS, J., and BENITO, C. 1985. Association of isozymes with a reciprocal translocation in cultivated rye (*Secale cereale* L.). Genetics, **109**: 177–193.

GIRALDEZ, R., and ORELLANA, J. 1979. Metaphase I bonds, crossing over frequency and genetic length of specific chromosome arms of rye. Chromosoma, **72**: 377–385.

GIRALDEZ, R., CERMEÑO, M. C., and ORELLANA, J. 1979. Comparison of C-banding pattern in the chromosomes of inbred lines and open pollinated varieties of rye. Z. Pflanzenzuecht. **83**: 40–48.

HENDERSON, S. A., and KOCH, C. A. 1970. Co-orientation by physical tension: a demonstration with experimentally interlocked bivalents. Chromosoma, **29**: 207–216.

KRAMER, H. H., and BLANDER, B. A. S. 1961. Orienting linkage maps in the chromosomes of barley. Crop Sci. **1**: 339–342.

LEWIS, K. R., and JOHN, B. 1963. Spontaneous interchange in *Chorthippus bruneus*. Chromosoma, **14**: 618–637.

MATHER, K. 1935. Reductional and equational separation of the chromosomes in bivalents and multivalents. J. Genet. **30**: 53–78.

NARASINGA RAO, P. S. R. L., and SYBENGA, J. 1984. Linear orientation of trivalents and quadrivalents in late metaphase I of pollen mother cells of pearl millet. Can. J. Genet. Cytol. **26**: 205–510.

NICKLAS, R. B., and KOCH, C. A. 1969. Chromosome micromanipulation. III. Spindle fiber tension and the reorientation of mal-oriented chromosomes. J. Cell Biol. **43**: 40–50.

ORELLANA, J., and GIRALDEZ, R. 1983. Metaphase I bound arms and crossing over frequency in rye. III. Non-chiasmate bonds in desynaptic plants. Heredity, **51**: 383–394.

——— 1984. Metaphase I bound arms and crossing over frequency in rye. IV. Triticale–rye hybrids. Heredity, **52**: 265–271.

ORELLANA, J., ALAMO, I., and GIRALDEZ, R. 1984. Metaphase I bound arms and crossing over frequency in rye. II. Inbred lines. Z. Pflanzenzuecht. **93**: 199–211.

RICKARDS, G. K. 1983. Orientation behaviour of chromosome multiples of interchange (reciprocal translocation) heterozygotes. Annu. Rev. Genet. **17**: 443–498.

SYBENGA, J. 1975. Meiotic configurations. Springer-Verlag, Berlin, Heidelberg, New York.

——— 1983. Rye chromosome nomenclature and homoeology relationships. A workshop report. Z. Pflanzenzuecht. **90**: 297–304.

SYBENGA, J., and VERHAAR, H. 1980. Chiasma patterns in a translocation derived duplication heterozygote of rye. Chromosoma, **80**: 147–162.

SYBENGA, J., and WOLTERS, A. H. G. 1972. The classification of the chromosomes of rye (*Secale cereale* L.): a translocation tester set. Genetica (The Hague), **43**: 453–464.

SYBENGA, J., VAN EDEN, J., VAN DER MEIJS, Q. C., and ROETERDINK, B. W. 1985. Identification of the chromosomes of the rye translocation tester set. Theor. Appl. Genet. **69**: 313–316.