Theor Appl Genet (1994) 89: 33–41
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Physical mapping of translocation breakpoints in rye by means of synaptonemal complex analysis

Received: 1 December 1993 / Accepted: 21 December 1993

Abstract A physical map including 40 translocation breakpoints has been constructed in rye by means of synaptonemal complex (SC) analysis of well-paired pachytene quadrivalents. The chromosome arms involved in such translocations were previously identified either from mitotic C-banding analysis or from the meiotic configurations observed in the progenies of crosses with a rye line having multiple chromosome rearrangements. The synaptonemal complexes formed by some translocation homozygotes were also analyzed, the relative pachytene SC length of their translocated chromosomes being compared to that observed in the corresponding translocation heterozygotes. In the translocations in which the position of the breakpoint could be well defined from mitotic C-banding analysis, a good correspondence between the relative position of the point showing partner exchange in the pachytene quadrivalents and the actual location of the breakpoint was established. It is concluded that the mapping of translocation breakpoints by SC analysis of pachytene quadrivalents provides a more accurate estimate of the position of the breakpoints than that obtained from mitotic C-banding analysis, due to the lack of evenly-distributed interstitial C-bands in most rye chromosomes. The distribution of the breakpoints along the chromosomes in relation to their spontaneous or induced origin is also discussed.

Key words Physical mapping
Translocation breakpoint · C-banding
Synaptonemal complex · Rye

Introduction

The utilization of deletions and translocations as reference points for locating genes or other DNA sequences in plant physical mapping requires previous knowledge of the location of the corresponding breakpoints. Terminal deletion breakpoints can be easily mapped by measuring mitotic chromosomes, but large deletion stocks are only available in polyploid plants, such as wheat, which can tolerate the loss of a chromosome segment (Werner et al. 1992; Gill et al. 1993; Kota et al. 1993). In contrast, translocations have been the chromosome rearrangements most extensively used in the cytogenetic mapping of diploid plants. In some species, such as maize, well-spread pachytene chromosomes with numerous structural features (chromomeres, centromeres and knobs) can be obtained and precise locations of translocation breakpoints can be established from light microscope analysis (McClintock 1930; Burnham 1962). However, the chromosomes of diploid *Triticeae*, such as barley and rye, are not amenable to pachytene analysis. Also, due to the low number and non-homogeneous distribution of their C-bands, most of the translocations in these species can only be assigned imprecisely to large chromosome segments (de Vries and Sybenga 1976; Linde-Laursen 1988).

The use of electron microscope analysis of pachytene synaptonemal complex (SC) spreads of translocation heterozygotes (Leblon et al. 1986; de Jong et al. 1989; Naranjo et al. 1989; Zickler et al. 1992; Alonso-Blanco et al. 1993, 1994), enables the precise location of the breakpoints in species with a scarcity of C-bands, thus increasing the number of cytological markers that can be used in physical maps by cytogenetic segregation analysis or by in-situ hybridization experiments.

In the present paper, the physical positions of the breakpoints of 20 translocations affecting all seven rye chromosomes have been determined by an analysis of the pachytene synaptonemal complexes (SCs) of the quadrivalents formed by the four chromosomes involved in the translocation in heterozygous plants. In order to evaluate the use of SCs in rye physical mapping, the relationship between the synaptonemal complex and mitotic karyotypes is analyzed. The distribution of the breakpoints within the karyotype is discussed in relation to the mode of origin of the different translocations.

Communicated by F. Salamini

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Materials and methods

Plant material

The translocations analyzed in this study originate from three different sources: (1) translocations *T240W*, *T242W*, *T248W*, *T273W*, *T282W*, *T305W*, *T306W*, *T501W* and *T850W* belong to the Translocation Tester Set (Wageningen, The Netherlands; Sybenga and Wolters 1972); (2) translocations *TR01*, *TR04*, *TR05*, *TR06*, *TR08*, *TR09*, *TR12*, *TR13*, *TR14* and *TR15* are from the cultivar 'Ailés', in which they appeared spontaneously, (3) translocation *TR03* also appeared spontaneously in the cultivar 'Snoopy'. All the translocation lines are maintained at the Departamento de Biología Funcional, Universidad de Oviedo, Spain.

A rye line (*CL1*), homozygous for translocations *T305W* (involving chromosomes 2R and 5R) and *TR01* (involving 4R and 6R), and double ditelocentric for chromosomes 1R and 3R, which had been produced previously in our laboratory, was used in the identification of the chromosomes involved in some translocations.

Mitotic and meiotic metaphase C-banding analysis

Root tips were immersed in tap water at 0 °C for 24 h, to shorten the chromosomes, and then fixed in acetic acid – alcohol 1 : 3. Anthers having PMCs at metaphase-I were fixed also in acetic acid – alcohol 1 : 3. The materials were maintained in the fixative for 1–4 months at 3–4 °C and then squashed and stained following the Giemsa C-banding technique described by Giraldez et al. (1979).

Synaptonemal complex analysis

The pachytene SCs were analyzed following the spreading and staining procedures of de Jong et al. (1989) as modified by Naranjo et al. (1989).

The SCs were studied in the electron microscope, photographed, and printed at a final magnification of ×7 000. The lengths of the SCs were measured on the photographic prints by means of a digitizing tablet connected to a computer.

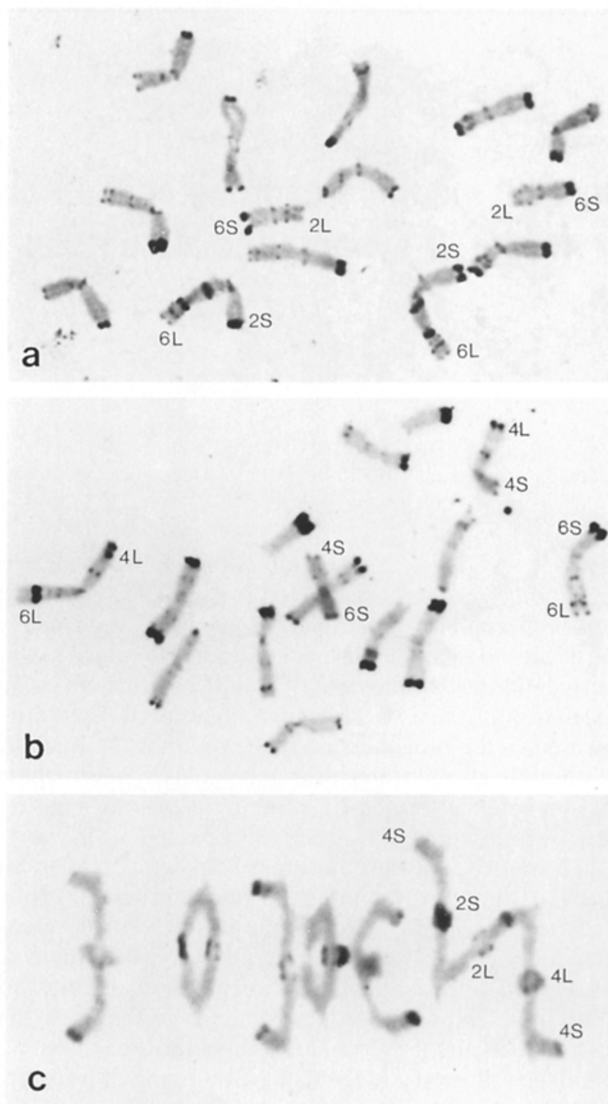

Fig. 1 a–c Some examples of mitotic and meiotic C-banded metaphases of the translocations analyzed. **a** Mitotic metaphase of a homozygote for translocation T242W; **b** mitotic metaphase of a heterozygote for translocation TR01, heterozygous for a centromere split of chromosome 3R; **c** metaphase-I of a heterozygote for translocation TR05

Results

Identification of the two chromosomes involved in each translocation and the arm location of the breakpoints

In most translocations, the identification of the chromosomes involved could be accomplished after the analysis of their C-banding pattern at mitosis. In the cases in which mitotic identification was questionable, the plants carrying the translocation were crossed with line *CL1*, and the chromosomes involved in the translocation were determined after analysis of the meiotic configurations of the progeny.

In most of the translocations studied, the chromosome arms carrying the breakpoints could be determined from the mitotic C-banding pattern of the translocated chromosomes or else were deduced from the presence of interstitial chiasmata in C-banded translocation quadrivalents. However, since most rye chromosomes have a small number of interstitial C-bands, this identification was not always possible. Nevertheless, the telomere constitutions of the two translocated chromosomes was determined in all cases, either from the analysis of their C-banding pattern at mitosis, from the analysis of C-banded quadrivalents at meiosis in plants heterozygous for the translocation and for specific C-bands, or from the analysis of the meiotic configurations of the offspring of the cross between the translocated plants and line *CL1*. Figure 1 shows examples of mitotic and meiotic C-banded chromosomes of some of the translocations studied. Table 1 shows the telomere constitutions of the translocated chromosomes of all translocations analyzed. The data concerning the Translocation Tester Set agree with the results of de Vries and Sybenga (1976) and Sybenga et al. (1990).

Knowledge of the telomere constitutions of the chromosomes involved in a non-complex reciprocal translocation excludes two possibilities concerning the arm location of the translocation breakpoints. For instance, in translocation TR05 (involving chromosomes 2R and 4R) the two

Table 1 Length (μ±SE) of the synaptonemal complex (SC) segments of the quadrivalents formed in pachytene cells of heterozygotes for the different translocations. For each translocation, segments I+II (or Sat.+I+II) and III+IV correspond to the SC formed by the two non-translocated chromosomes, respectively (see Fig. 2)

Trans-location	Telomere constitution of the translocated chromosomes	Quadrivalent segments						SC length of bivalent 1R	SC mean length of the remaining bivalents	Number of pachytene cells
		Sat.	Sat. (Int)	I	II	III	IV			
T240W	3RS-5RL/3RL-5RS	–	–	21.0 ± 2.6	60.2 ± 3.6	52.9 ± 3.1	27.0 ± 2.2	66.3 ± 3.0	73.3 ± 3.4	6
T242W	2RS-6RL/6RS-2RL	–	–	26.0 ± 1.4	46.0 ± 2.3	0.0 ± 0.0	74.5 ± 3.5	62.2 ± 2.1	70.4 ± 3	7
T248W	1RS-6RL/1RL-6RS	–	4.9 ± 0.6	9.3 ± 1.1	74.8 ± 4.7	17.7 ± 1.5	81.1 ± 5.6	–	96.1 ± 6.1	7
T273W	1RS-5RS/1RL-5RL	10.8 ± 0.6	–	9.6 ± 2.8	60.0 ± 1.7	52.5 ± 3.5	36.5 ± 3.5	–	85.1 ± 5.8	5
T282W	5RS-7RS/5RL-7RL	–	–	11.3 ± 2.6	52.7 ± 4.1	35.2 ± 4.4	36.4 ± 3.4	59.6 ± 0.7	68.6 ± 0.7	10
T305W	2RS-5RL/2RL-5RS	–	–	17.1 ± 1.8	64.7 ± 4.6	41.3 ± 3.2	45.9 ± 3.4	78.1 ± 5.2	84.1 ± 5.3	5
T306W	1RS-6RS/1RL-6RL	8.9 ± 1.4	–	9.9 ± 1.3	54.7 ± 10.8	23.8 ± 3.5	61.9 ± 12.4	–	76.9 ± 8.9	6
T501W	4RS-5RL/4RL-5RS	–	–	14.2 ± 2.1	60.4 ± 3.9	42.5 ± 1.6	31.1 ± 2.5	66.5 ± 4.3	72.3 ± 3.1	9
T850W	1RS-4RS/1RL-4RL	–	7.7 ± 0.6	6.2 ± 0.8	83.8 ± 5.4	50.2 ± 3.1	56.7 ± 2.8	–	104.1 ± 4.6	10
TR01	4RS-6RS/4RL-6RL	–	–	19.3 ± 2.2	62.1 ± 5.1	39.6 ± 3.1	40.3 ± 2.6	64.8 ± 5.2	75.1 ± 6.0	7
TR03	4RS-5RS/4RL-5RL	–	–	17.1 ± 2.1	61.7 ± 3.8	44.3 ± 4.2	27.3 ± 2.2	58.9 ± 5.0	73.3 ± 3.1	5
TR04	1RS-4RL/1RL-4RS	9.2 ± 0.7	–	15.0 ± 2.3	46.0 ± 1.6	24.0 ± 2.7	49.6 ± 1.5	–	75.8 ± 2.4	7
TR05	2RS-4RS/2RL-4RL	–	–	26.4 ± 2.2	53.2 ± 1.5	44.7 ± 2.9	33.8 ± 0.9	65.5 ± 2.4	71.5 ± 5.0	5
TR06	3RS-4RS/3RL-4RL	–	–	21.3 ± 1.4	44.8 ± 3.2	30.9 ± 3.3	34.4 ± 3.7	55.6 ± 2.7	63.4 ± 3.5	5
TR08	4RS-7RS/4RL-7RL	–	–	24.8 ± 2.0	48.9 ± 1.6	39.0 ± 3.2	36.9 ± 1.5	67.4 ± 4.6	76.4 ± 1.9	7
TR09	4RS-5RL/4RL-5RS	–	–	23.4 ± 2.1	35.2 ± 1.5	28.4 ± 3.2	30.9 ± 1.8	50.4 ± 3.4	57.8 ± 2.5	8
TR12	2RS-6RS/2RL-6RL	–	–	21.8 ± 1.3	54.7 ± 3.3	49.3 ± 3.7	32.6 ± 2.0	67.8 ± 4.6	75.5 ± 4.2	6
TR13	3RS-7RL/3RL-7RS	–	–	25.1 ± 2.5	43.5 ± 3.9	27.8 ± 1.8	36.8 ± 3.5	65.7 ± 4.9	69.0 ± 4.7	6
TR14	2RS-7RL/2RL-7RS	–	–	25.9 ± 3.4	51.1 ± 2.7	30.6 ± 3.3	42.3 ± 3.0	63.9 ± 2.7	74.7 ± 2.0	6
TR15	4RS-5RL/4RL-5RS	–	–	23.9 ± 3.6	66.1 ± 5.3	32.4 ± 5.5	55.2 ± 3.2	72.0 ± 5.6	80.7 ± 5.0	5

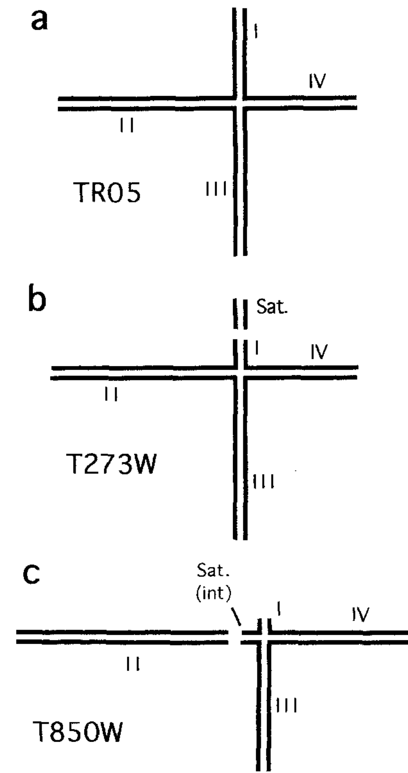

Fig. 2a–c Schematic representation of three examples of translocations indicating the segments that can be distinguished in the corresponding SC quadrivalents. The normal, non-translocated chromosomes are constituted by segments I+II (or Sat+I+II) and III+IV (see Table 1). **a** Translocation TR05 (involving 2R and 4R) in which only four segments can be distinguished. **b, c** Translocations T273W (1R and 5R) and T850W (satellite of 1R and 4R) in which five segments can be distinguished

translocated chromosomes have the telomere constitutions 2RS-4RS (small translocated chromosome) and 2RL-4RL (long translocated chromosome), indicating that the translocation breakpoints are located either in the arms 2RS and 4RL, or in the arms 2RL and 4RS, the two other possibilities, 2RS and 4RS or 2RL and 4RL, being excluded.

SC analysis and determination of the physical location of the translocation breakpoints

The technique of SC spreading in rye does not allow the identification of the centromeres. Therefore, only five or four segments can be distinguished in the pachytene quadrivalent, depending on whether or not chromosome 1R (carrying the satellite) is involved in the translocation (Fig. 2). However, the length of the four chromosomes involved in the quadrivalent can be individually determined and the relative position of the translocation breakpoints can be ascertained. Table 1 shows the length of the identified quadrivalent segments in the different translocations studied. Figure 3 shows the spread SCs from a pachytene nucleus of one of the translocation heterozygotes analyzed.

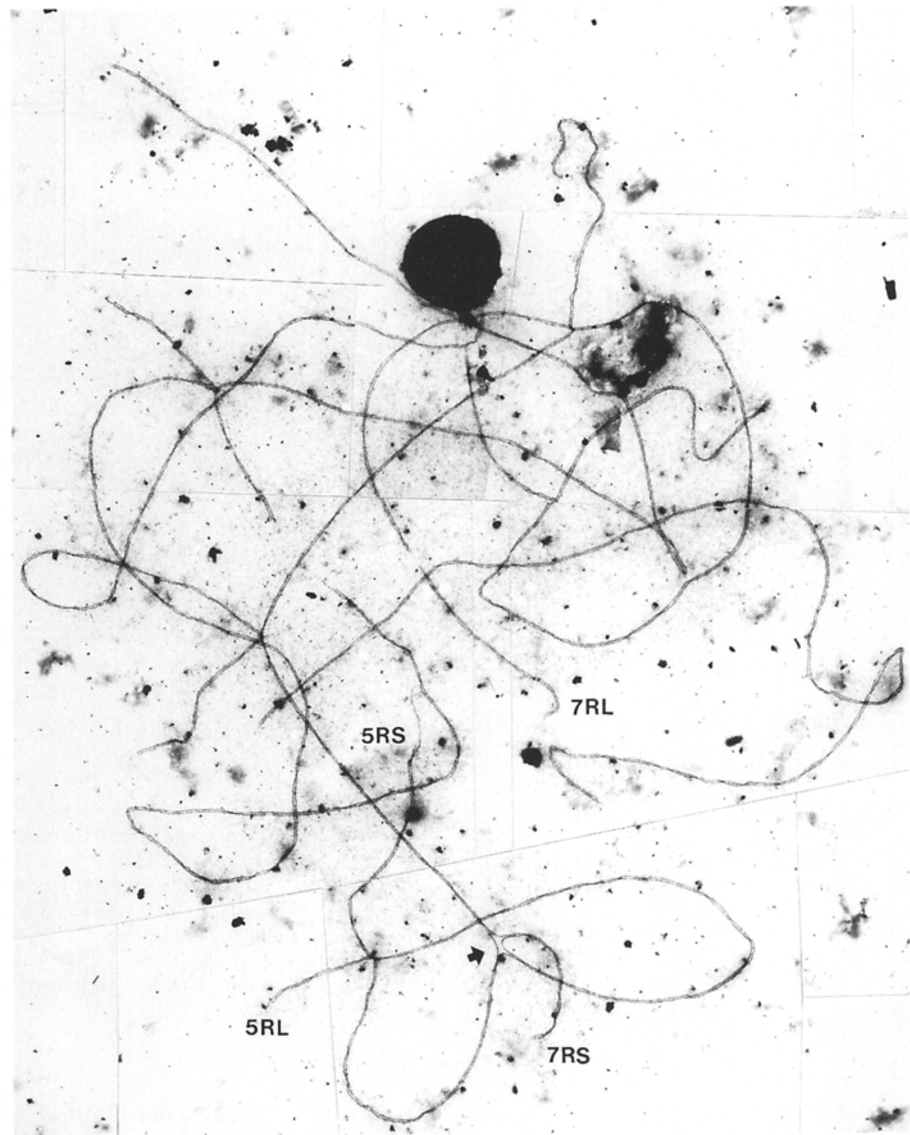

Fig. 3 Electron micrograph of the spread synaptonemal complexes from a pachytene nucleus of a heterozygote for translocation T282W. The *arrow* shows the partner exchange (translocation breakpoint)

Considering the data obtained from the SC analysis, together with the data obtained from the C-banding mitotic and meiotic analysis, the most probable physical location of the translocation breakpoints can be deduced, even in cases in which the chromosome arms involved in the translocation were not known. For instance, considering the lengths of the four SC segments of the quadrivalent of translocation TR05 (Table 1), the lengths of the four chromosomes involved in such a quadrivalent are 79.6 μ (I + II), 78.5 μ (III + IV), 60.1 μ (I + IV) and 97.9 μ (II + III) respectively. Since chromosomes 2R and 4R (involved in translocation TR05) are not very different in length (see below), the normal non-translocated chromosomes are the ones constituted by segments I + II and III + IV respectively, the small translocated chromosome is constituted by segments I + IV and the long translocated chromosome by segments II + III. Since the telomeres of the translocated chromosomes are 2RS – 4RS and 2RL – 4RL, respectively (Table 1), there would be four possible interpretations concerning the correspondence of the segments I, II, III and IV in the pachytene quadrivalent to specific chromosome ends (Fig. 4). Now, considering a relative length of 47% for chromosome arm 2RS and 38% for 4RS (Giraldez et al. 1979), the best interpretation is the one in which chromosome I + II is chromosome 2R and chromosome III + IV is chromosome 4R; segments I, II, III and IV corresponding to chromosome ends 2RS, 2RL, 4RL and 4RS, respectively (Fig. 4a). This is also in agreement with the mitotic C-banding data from which it could be concluded that the smallest translocated chromosome had the telomere constitution 2RS-4RS.

The assignment of quadrivalent SC segments to specific chromosome ends was easier in the translocations in which the chromosome arms involved were previously known and in those involving chromosome 1R (see Fig. 2b, c), since the number of possible interpretations was reduced. Nevertheless, in five of the translocations analyzed, the location of the breakpoints was not unequivocally deter-

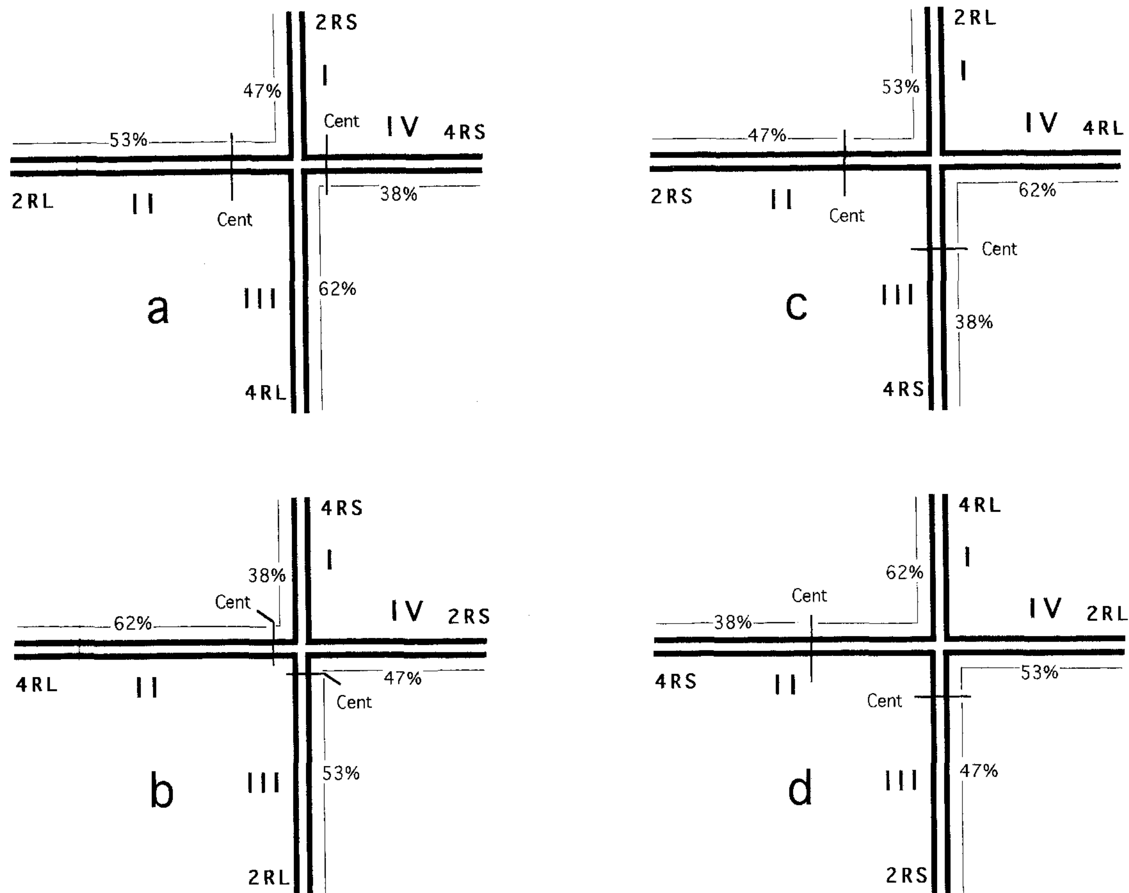

Fig. 4 a–d Diagrammatic representation of the four possible pachytene quadrivalent constitutions of translocation TR05, taking into account the length of the quadrivalent segments. After locating the centromeres from mitotic arm ratios (see text for explanation), the most probable quadrivalent constitution is the one indicated in **a** (centromeres in opposite arms of the quadrivalent)

mined, a unique interpretation of the quadrivalent not being reached.

Figure 5 shows the relative locations of the breakpoints of the 20 translocations analyzed.

Relative length of the pachytene SCs formed by the different rye chromosomes

The presence of the nucleolar-organizing region allowed the identification of the SCs formed by bivalent 1R in all cells. Also, the length of the SCs corresponding to the normal non-translocated chromosomes involved in each quadrivalent could be deduced. The relative length of the SCs corresponding to bivalent 1R and to the chromosomes involved in the different translocations are summarized in Table 2. The results indicate that the shortest SC is the one formed by chromosome 1R, followed by that of chromosome 7R, differences between the SCs, corresponding to the remaining chromosomes (2R, 3R, 4R, 5R and 6R) being smaller.

Comparison between the SCs formed by specific chromosomes in translocation homozygotes and heterozygotes

In order to ascertain whether the relative length of the SC formed by a specific chromosome depends on the meiotic configuration in which it is involved, the relative lengths of the SCs corresponding to chromosome 1R when forming a bivalent or when being involved in a quadrivalent were compared. The mean relative length of the SC of chromosome 1R in plants in which this chromosome formed a bivalent (12.81 ± 0.94) was slightly smaller than the one derived from quadrivalents in the plants in which this chromosome was involved in the translocation (13.45 ± 0.93), and this difference was significant ($t = 3.48$; $df = 129$; $P < 0.001$).

The analysis of the pachytene SCs of plants homozygous for translocations *T240W*, *T242W*, *T282W*, *T501W*, *T850W* and *TR01* provided a different approach to this problem. In these translocation homozygotes, the two pairs of translocated chromosomes form two bivalents which are very different in length, their corresponding SCs being easily identified. Table 3 shows the relative lengths of the SCs formed by the two translocated chromosomes of these ho-

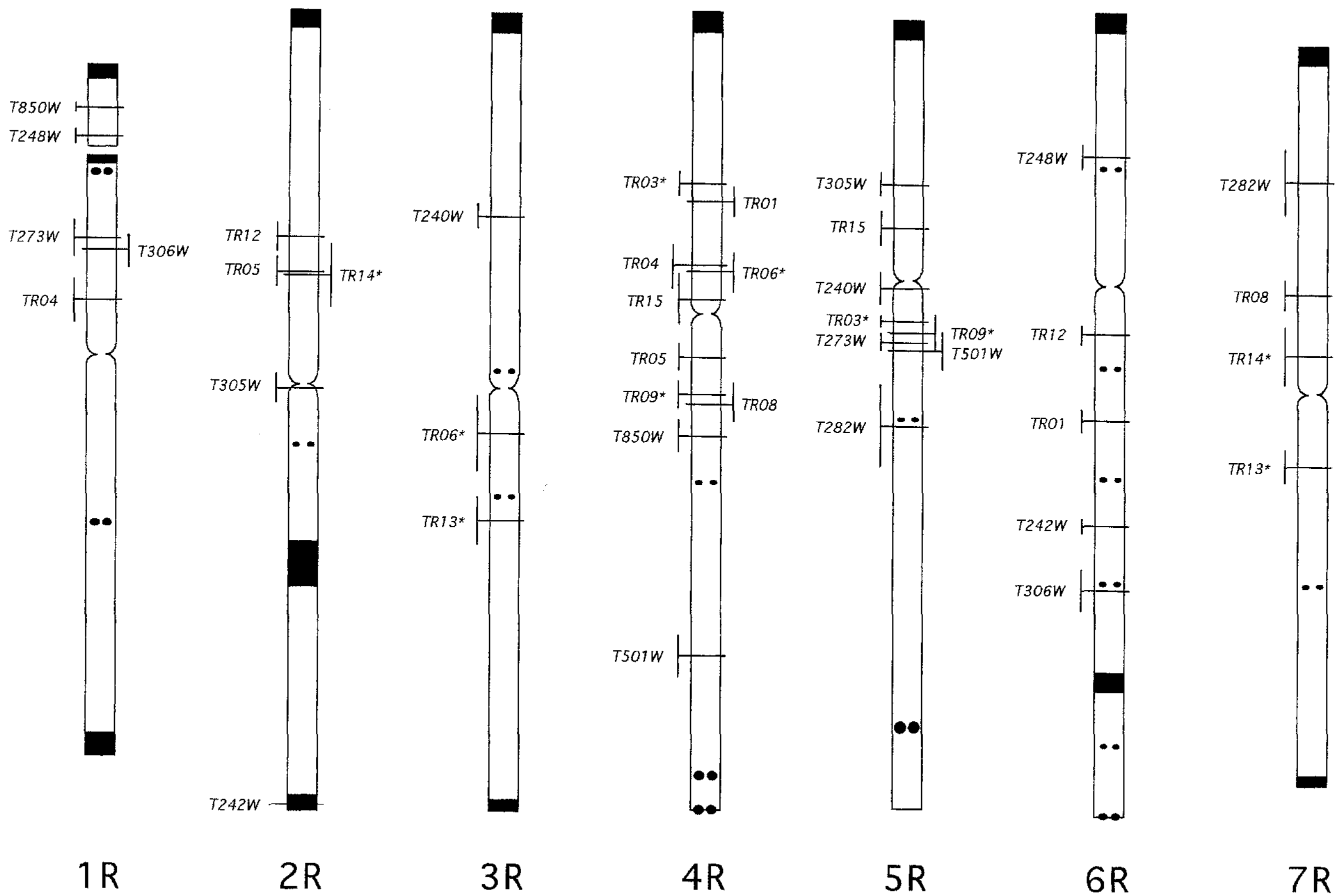

Fig. 5 Physical map (relative positions) of the breakpoints of the 20 translocations analyzed (standard errors are indicated by *vertical bars*). The length of the different chromosomes is proportional to the values indicated in Table 2. The position of the translocation breakpoints indicated by an *asterisk* was not unequivocally determined (see text)

Table 2 The relative length (%±SE) of the SCs corresponding to the chromosomes involved in the different translocations, derived from the analysis of the corresponding pachytene quadrivalents

Translocation	Chromosome						
	1R	2R	3R	4R	5R	6R	7R
T240W	12.76 ± 0.34	–	15.56 ± 0.73	–	15.34 ± 0.05	–	–
T242W	12.72 ± 0.20	15.17 ± 0.15	–	–	–	14.66 ± 0.29	–
T248W	13.35 ± 0.18	–	–	–	–	14.79 ± 0.20	–
T273W	13.64 ± 0.69	–	–	–	14.92 ± 0.26	–	–
T282W	12.69 ± 0.08	–	–	–	15.16 ± 0.37	–	13.69 ± 0.45
T305W	13.41 ± 0.50	14.92 ± 0.25	–	–	13.99 ± 0.19	–	–
T306W	13.44 ± 0.56	–	–	–	–	15.44 ± 0.62	–
T501W	13.13 ± 0.43	–	–	14.79 ± 0.32	14.71 ± 0.48	–	–
T850W	13.45 ± 0.29	–	–	14.77 ± 0.21	–	–	–
TR01	12.34 ± 0.43	–	–	15.52 ± 0.49	–	15.27 ± 0.28	–
TR03	11.69 ± 0.67	–	–	15.62 ± 0.41	14.17 ± 0.75	–	–
TR04	13.42 ± 0.19	–	–	14.10 ± 0.17	–	–	–
TR05	12.92 ± 0.45	15.68 ± 0.32	–	15.48 ± 0.34	–	–	–
TR06	12.62 ± 0.10	–	14.82 ± 0.09	15.01 ± 0.14	–	–	–
TR08	12.82 ± 0.49	–	–	14.52 ± 0.36	–	–	14.12 ± 0.28
TR09	12.55 ± 0.31	–	–	14.84 ± 0.12	14.69 ± 0.17	–	–
TR12	12.81 ± 0.22	14.54 ± 0.30	–	–	–	15.49 ± 0.21	–
TR13	13.92 ± 0.47	–	14.50 ± 0.21	–	–	–	13.71 ± 0.47
TR14	12.47 ± 0.45	15.06 ± 0.64	–	–	–	–	14.21 ± 0.27
TR15	12.56 ± 0.32	–	–	15.28 ± 0.42	15.66 ± 0.40	–	–
Relative average to total length = 100%	12.70	14.80	14.69	14.72	14.56	14.85	13.68

Table 3 The comparison between the relative lengths (%) of the pachytene synaptonemal complexes (SC) corresponding to the translocated chromosomes in heterozygotes and homozygotes for six translocations

Trans-location	Long/short translocated chromosomes	Mean relative SC length (% ± SE) of the long translocated chromosome			Mean relative SC length (% ± SE) of the short translocated chromosome			Number of pachytene cells	
		Trans-location hetero-zygote	Trans-location homo-zygote	t-test	Trans-location hetero-zygote	Trans-location homo-zygote	t-test	Trans-location hetero-zygote	Trans-location homo-zygote
T240W	3RL-5RL/3RS-5RS	21.7 ± 0.6	20.8 ± 0.3	0.95 n.s.	9.2 ± 0.2	9.3 ± 0.3	0.32 n.s.	6	3
T242W	2RS-6RL/6RS-2RL	20.5 ± 0.2	21.5 ± 0.2	3.53 **	9.4 ± 0.2	9.1 ± 0.3	0.53 n.s.	11	10
T282W	5RL-7RL/7RS-5RS	18.7 ± 0.3	19.4 ± 0.7	0.26 n.s.	10.1 ± 0.4	10.0 ± 0.3	0.13 n.s.	10	13
T501W	4RL-5RS/4RS-5RL	20.5 ± 0.6	20.9 ± 0.5	0.51 n.s.	9.0 ± 0.6	8.9 ± 0.2	0.21 n.s.	9	8
T850W	1RL-4RL/1RS-4RS	19.5 ± 0.3	19.3 ± 1.0	0.24 n.s.	8.7 ± 0.3	8.6 ± 0.3	0.14 n.s.	10	2
TR01	4RL-6RL/4RS-6RS	19.4 ± 0.5	18.6 ± 0.1	1.21 n.s.	11.4 ± 0.3	10.6 ± 0.2	1.64 n.s.	7	5

n.s., non-significant; **, $P < 0.001$

mozygotes, compared to the ones formed by the same chromosomes in the corresponding translocation heterozygotes (segments I+IV and II+III in Table 1). Except in one case, differences were not significant.

Discussion

In this study, the physical location of the translocation breakpoints in the chromosomes has been ascertained by assuming that the relative lengths of the different chromosomes, as well as that of the different segments along each chromosome at mitosis, are maintained in the corresponding pachytene SCs. While there seems to be a general agreement about the correspondence of SC karyotypes to those obtained by mitotic or meiotic chromosome analysis in different organisms (Moses et al. 1975; Gillies 1981; Kaebling and Fechheimer 1983; Wandall and Svendsen 1983), detailed quantitative analyses have revealed small differences usually restricted to the arm ratios of particular chromosomes (Moses et al. 1977; Solari 1980; Sherman and Stack 1992; Jones and Azkue 1993). Rye chromosomes are very similar in length, and, except for the presence of the satellite that defines chromosome 1R, their SCs do not show structural characteristics. Within and between-plants differences in the relative length of the SC formed by a specific chromosome are found, which can depend on small differences in contraction rate between chromosomes, or on differences in the amount of telomere C-heterochromatin (Gillies and Lukaszewski 1989; de Jong et al. 1989; Naranjo et al. 1989). Some authors have ordered the rye chromosomes according to their relative SC lengths in structurally normal karyotypes (Abirached-Darmency et al. 1983; Gillies 1985); however, since they could not distinguish them from one another, the differences in relative SC lengths obtained were probably overestimated due to the accumulation of small errors.

In the present study, the SC relative lengths of the different rye chromosomes were deduced from the measurements of the quadrivalents in which they were involved. The possible existence of delayed pairing around the translocation breakpoint (de Jong et al. 1989) could result in contraction differences between the quadrivalent and the bivalents, which in turn could lead to an overestimation of the relative lengths of the chromosomes involved in the quadrivalents. The results obtained in the present work, using almost-completely-paired pachytene cells, suggest that these differences, if they exist, are small. On one hand, chromosome 1R was only around 5% shorter when forming bivalents than when involved in the quadrivalent (Table 2). On the other hand, the comparison between the relative SC lengths of the translocated chromosomes in translocation homozygotes and heterozygotes (Table 3) indicates that the SC length formed by a specific chromosome does not depend on the meiotic configuration in which it is involved (bivalent or quadrivalent). It can be concluded that the relative SC lengths estimated for the seven rye chromosome pairs agree with those obtained previously from mitotic C-banding analysis (Giraldez et al. 1979).

Regarding the relative centromere position (arm ratio), studies on different organisms that allow a preservation of the centromeres in SC spreading have suggested only some localized discrepancies affecting specific chromosomes (Solari 1980; Gillies and Cowan 1985; Sherman and Stack 1992). In rye no spreading technique preserves the centromeric regions, but in serial reconstruction of early meiotic nuclei Abirached-Darmency et al. (1983) observed a good correlation between the SC arm ratio of pachytene bivalents and the light microscopic data of meiotic and mitotic chromosomes. On the other hand, SC analyses of ditelocentric heterozygotes (centromere splits of chromosomes 1R and 3R; Alonso-Blanco et al. 1993, 1994) and a secondary trisomic carrying an extra isochromosome for 6RS (unpublished data), have shown a good correspondence between the arm ratios of the pachytene SC and those of the corresponding mitotic chromosomes.

Although the SC analysis was carried out in cells containing completely-paired chromosomes, a variation in the relative position of the partner exchange within the quadrivalents was observed (Fig. 5), suggesting the possible existence of non-homologous pairing around the transloca-

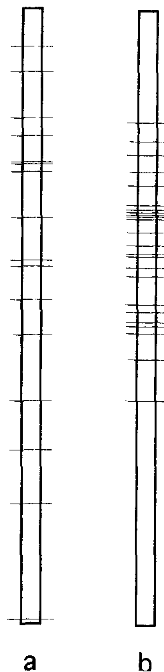

Fig. 6 a, b The within-chromosome relative location (pooled) of X-ray-induced translocation breakpoints **a** compared to that of spontaneous ones **b**

tion breakpoints. As was previously noted in maize translocations (Burnham 1962), there is no obvious relationship between the extent of this variation and the particular chromosomes or chromosome regions in which the breakpoints are located. In spite of this variation, it can be concluded that the average location of the partner exchanges within the quadrivalents represents a good estimation of the actual position of the translocation breakpoints since, in cases in which the location of a specific breakpoint could be determined from the analysis of C-banded mitotic chromosomes, a good agreement with the relative position of the partner exchange deduced from SC analysis was obtained. For instance, from mitotic analysis, the breakpoints of the four translocations involving chromosome arm 6RL were assigned to specific segments defined by the six C-bands present in this arm, the SC analysis of these translocation heterozygotes locating the partner exchanges in relative positions corresponding to the same chromosome segments (Figs. 1a,b and 5).

In this study, 40 translocation breakpoints have been assigned to small segments of 13 out of the 14 rye chromosome arms. The 20 translocations examined can be classified in two groups according to their origin. Twelve (TR01–TR15, and T282W) arose spontaneously in natural populations, while the remaining eight translocations were obtained after X-ray treatment of immature pollen (Sybenga and Wolters 1972). The translocations from both groups are unevenly distributed among the seven chromosomes. All the translocations belonging to the Wageningen tester set involve at least one of chromosomes 1R, 5R and 6R, probably because these chromosomes are the most favorable for originating readily-recognizable translocated chromosomes at mitosis, a character that was selected for in the construction of the tester set. By contrast, chromosome 4R was involved in eight out of the ten translocations of spontaneous origin derived from cv 'Ailés' and in the one derived from cv 'Snoopy'. In a previous study of the chromosomes involved in different translocations of cv 'Ailés' using C-banding and isozyme analyses, Figueiras et al. (1990) found that chromosome 4R was also preferentially involved, suggesting that transposon activity could be the reason for this behaviour.

As shown in Fig. 6, the distributions of the breakpoints within the chromosomes are also different in these two groups. The breakpoints of X-ray-induced translocations are scattered along the entire chromosome length, while those of the spontaneous translocations are clustered in medium and proximal regions. Again, the selection for readily-recognizable translocated chromosomes at mitosis in the construction of the tester set can explain the differences in the distribution of their breakpoints. The presence of one large and one small chromosome in most of these translocations would almost certainly require the existence of one distal and one proximal chromosome break. On the other hand, distal breakpoints would favor the existence of interstitial chiasmata in the quadrivalents formed in meiocytes of translocation heterozygotes (frying pan and figure eight-shaped configurations), and therefore a high frequency of unbalanced gametes. Consequently, the absence of distal breakpoints in the spontaneous translocations could be a result of their differential elimination.

Acknowledgments This work has been supported by grant AGR91-1305 of the CICYT, Spain. E. A. and P. G. G. are recipients of salary fellowships from the FICYT, Principado de Asturias, Spain, and C.A.-B. is recipient of a fellowship from the Ministerio de Educación y Ciencia, Spain. The authors wish to thank J. Sybenga, J. Orellana and A. Luckaszewski for kindly providing some of the translocations.

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