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Mapping seed storage protein loci *Sec-1* and *Sec-3* in relation to five chromosomal rearrangements in rye (*Secale cereale* L.)

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Summary. Linkage relationships were established between the secalin loci, *Sec 1* (40-K gamma and omega secalins, homologous to the wheat gliadins) and *Sec 3* (HMW = high-molecular-weight secalins, homologous to the wheat HMW glutenin subunits), and five chromosomal rearrangements involving chromosome *1R* of rye (*Secale cereale* L.). These were: interchanges T273W (*1RL/5RS*), T306W (*1RS/5RL*), and T850W (*1RS/4RL*), Robertsonian centromere split Rb1RW and the interchanged Robertsonian split Rb2R/248W. The analysis established the linkage relationships between the secalin loci and the breakpoints of the rearrangements, in addition to the quantitative effects of the rearrangements on the linkage. *Sec-1* is located in the satellite at a position at least 2.5 cMorgan from the proximal border of the terminal C-band, and about 30 cMorgan from the nucleolar organizing region (NOR). The locus is also physically closer to the terminal C-band than to the NOR, but not as much as corresponds with the map distances. Similarly, the physical distance between *Sec-3* and the centromere is greater than corresponds with the recombination frequency (0%–9%). Although overall recombination in *1RL* remains the same, recombination between the centromere and *Sec-3* is greatly reduced in the Robertsonian split combined with the interchange. This is not the case with the single Robertsonian split.

Key words: *Secale* – Secalin loci – Mapping – Rearrangements – Chromosome *1R*

Introduction

Two major multigenic loci encoding prolamin storage proteins are present on the group 1 chromosomes of wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.). One of these (called *Glu-1* in wheat and *Sec-3* in rye) encodes the high-molecular-weight prolamin subunits (HMW subunits of glutenin in wheat and HMW secalins in rye). The second locus encodes a mixture of omega gliadins, gamma gliadins, and low-molecular-weight (LMW) subunits of glutenin in wheat (*Gli-1*), and omega secalins and the mol.wt. 40,000 (40 K) gamma secalins in rye (*Sec-1*). There are striking similarities in the locations of these loci in the two species and in the properties of their products (Kreis et al. 1985).

Lawrence and Shepherd (1981) found no statistically significant linkage between the *Glu* and *Gli* loci on chromosome *1B* of wheat (48.8% recombination). Payne et al. (1982), however, concluded that there was loose but statistically significant linkage in a different material (average 43.3% recombination), and the same was observed for the corresponding genes in chromosomes *1A* and *1D*.

By telocentric mapping, Payne et al. (1982) found 8.9% recombination between the HMW locus in the long arm and the centromere for all three chromosomes *1A*, *1B*, and *1D*. Corrected for reduced meiotic pairing of the telocentrics, the estimate of recombination increased to 9.5%.

Analyses carried out with telocentrics may lead to underestimaton of the genetic map distance (Fu and Sears 1973), although Dvorak and Appels (1986) suggest that this may at least partly depend on the chromosomal combination involved.

From the absence of proteins coded by the *Gli-1* locus in a wheat line lacking the satellite on the short arm of chromosome *1B*, Payne et al. (1984) concluded that the *Gli-1* genes are located in the satellites of the group 1 chromosomes. A second locus encoding LMW subunits of glutenin (*Glu-2*) in the short arm of chromosome *1B* is absent from the other group 1 chromosomes of wheat and from rye.

The map distances for rye are comparable. Shewry et al. (1984) reported statistically significant linkage with 40.8% recombination between the omega (*Sec 1*) and HMW (*Sec 3*) secalin loci in an F_2 of 466 plants of a cross between two weedy ryes, formerly described as *S. turkestanicum* and *S. dighoricum*. Singh and Shepherd (1984) used a line of the wheat variety Chinese Spring with a *1RL/1DS* translocation, crossed with a wheat line with the *1D* chromosomes substituted by complete *1R* chromosomes. Among testcross progeny of 479 plants, 70 (14.6%) were aneuploids, resulting from pairing failure (37.8%) of the *1RL* rye arms. The parental types were represented by 196 and 194 plants, respectively, and the recombinants by 9 and 10 plants. Neglecting aneuploids, a recombination of $4.46\% \pm 1.04\%$ was estimated between the HMW locus and the centromere.

Recombination between *Sec-1* and *Sec-3* of 36.8% was reported by Lawrence and Appels (1986), which is less than found by Shewry et al. (1984). No recombination between the omega and 40-K gamma secalin genes has ever been reported, and they are assumed to occupy the same locus. The recombination percentages in rye are somewhat smaller than in wheat.

In the present study the recombination between the loci of *Sec-1* and *Sec-3* and a number of chromosomal rearrangements was analyzed. The purpose was to estimate recombination between these loci and the breakpoints of the rearrangements, including the centromere, and to make a better estimate of the physical location of the loci. Another purpose was to study the effects of the rearrangements, which include interchanges and telocentrics, on recombination.

Materials and methods

Five rearrangements were used. They were crossed with rye inbred line 129 developed by Nilsson-Ehle in Sweden in the 1920s, and kindly made available by A. Lundquist of the University of Lund. This line has patterns of secalins that can be distinguished from those of most of the rearranged stocks used. Testcrosses were made with the most favorable line as the recurrent plant.

There were three interchanges (reciprocal translocations) and two Robertsonian (centromere) splits, one of which carried a reciprocal translocation. These are diagrammatically shown in Fig. 1, both as somatic chromosomes with the (approximate) breakpoints and the preliminary locations of the secalin loci indicated, and as meiotic pairing configurations.

T273W (1RL/5RS) (Fig. 1a)

Only segregation for the interchange and *Sec-3* (HMW secalins) could be analyzed. Data on meiotic behavior were available from earlier studies (Sybenga 1975). The break in *1RL* had earlier been found to be (physically) slightly proximal to the middle of the arm. The break in *5RL* is more distal. In meiosis all four end segments of the interchange complex tended to have at least one chiasma, but chiasmata were the least frequent in the *1R/5R* translocated arm.

T306W (1RS/6RL) (Fig. 1b)

Only segregation for the interchange and *Sec-1* (40-K gamma secalins) could be analyzed. The break is in the short arm of *1R*, proximal to the NOR.

T850W (1RS/4RL) (Fig. 1c)

Segregation for the interchange, *Sec-3* (HMW secalins), and *Sec-1* (omega secalins) was analyzed in the testcross with the interchange. Segregation for the interchange, *Sec-3* (HMW secalins), and *Sec-1* (40-K gamma secalins) was analyzed in the testcross with 129. The break is distally located in the satellite of *1RS*, just proximal to the terminal C-band. All or almost all of the distal C-band of *1RS* has been translocated to *4RL*, in exchange for a much larger segment from *4RL*. The small *4R/1R* chromosome carries pronounced C-bands at both ends. A very small band in the translocated chromosome *1R/4R*, located approximately where the terminal *1RS* C-band could be expected, many be a small proximal segment of this band. Alternatively, it may be a very minor interstitial band translocated from *4RL*. Meiosis was analyzed in one heterozygote in the testcross progeny.

Rb1RW (Fig. 1d), available in two genotypes

The F_1 involving genotype 016 was testcrossed with line 129 to establish the segregation of the rearrangement and *Sec-1* (omega secalins). The F_1 involving genotype 029 was testcrossed with 129 to establish the segregation of the rearrangement and the combination of *Sec-3* (HMW secalins) and *Sec-1* (40-K gamma secalins).

Rb1R/T248W (1RS/6RS) (Fig. 1e)

Segregation of the rearrangement, *Sec-3* (HMW secalins), and *Sec-1* (omega and 40-K gamma secalins) was studied. Meiosis was analyzed in two heterozygotes from one of the testcross progenies.

The recombination percentages have not been converted to map distance, e.g., using the Kosambi function, as in most other reports. Map distances are additive, which is of interest when different recombination percentages are combined into a map. However, map distances are not of special interest for single intervals, as in this report, and recombination percentages are more realistic.

The parent on which segregation took place was used as the female in the testcross because of the double dose of the maternal genes in the endosperm, which enhances their expression. Not all rearrangements had genotypes suitable for studying both *Sec* loci. In addition, some combinations caused difficulties during analysis (particularly those involving 40-K gamma secalins) or suffered from reduced germination.

Single testcross seeds were cut in half. The embryo half was kept in Wageningen, germinated, and was classified for the rearrangement in the seedling stage, grown in small clay pots. Root tips were pretreated with 1-bromonaphthalene, fixed, and macerated in 1 *N* HCl at 59 °C for 12 min, then stained according to Feulgen. Some root tips were C-banded, especially in the families involving T850W and Rb1R/248, but in most cases no C-band polymorphism was found, and in others it was not sufficiently distinct for reliable classification of large numbers of plants.

Meiosis was analyzed in acetocarmine-stained PMCs after 1:3 acetic alcohol fixation.

Analysis of secalins

The non-embryo halves of the seeds were extracted at the Rothamsted Experimental Station, as described by Shewry et al.

(1983). Secalin patterns were determined by sodium dodecyl sulphate electrophoresis (SDS-PAGE).

Two basic systems were used, a Laemmli (1970) system with TRIS/glycine buffers and a system with TRIS/borate buffers (Bunce et al. 1985). These systems were sometimes modified by the addition of 4 *M* urea to the Laemmli (1970) system or 2 m*M* dithiothreitol to either system, to give optimum resolution of the secalin alleles segregating in the different crosses. Even so, it was not possible to score for segregation of 40-K gamma secalins and omega secalins in the same cross.

Results

T273W (Fig. 1a, Table 1)

Recombination between *Sec-3* (HMW secalins) and the breakpoint of the interchange was 11.3%, in a total of 62 testcross progeny from three pooled populations (85611, 86418, and 86421). The frequency of chiasmate association of the segment distal to the break in translocation heterozygotes was rather high. Chiasmata in the interstitial segment between the break and the centromere were not observed with certainty (Sybenga 1975). The recombination frequency between the break and the *Sec-3* locus corresponded with that between the centromere and *Sec-3* in normal material, as summarized by Kreis et al. (1985). It was also very similar to the distance between the centromere and the *Glu-1* (HMW glutenin) loci on the group 1 chromosomes in wheat. The *Sec-3* locus is apparently distal to the break of T273W in *1RL*, and recombination was not reduced by the break.

T306W (Fig. 1b, Table 2)

In a total population of 104 testcross progeny seeds from five families (87357, 87358, 87359, 87360, 87361), a recombination of 24% for the rearrangement and *Sec-1* (40-K gamma secalins) was observed.

T850W (1RS/4RL) (Fig. 1c, Tables 3 and 4)

There were two groups of testcross families. In one group the interchange parent was the recurrent parent and segregation for *Sec-1* (omega secalins) and the interchange could be analyzed (86632–86636). In three of these (86634, 86635, 86636), *Sec-3* (HMW secalins) also segregated. In the second group of five families, line 129 was the recurrent parent, and segregation for *Sec-1* (40-K gamma secalins) and *Sec-3* (HMW secalins) and the interchange could be analyzed (87362–87366).

From the segregation among 134 progeny plants of the first group scored only for the interchange and *Sec-1*, a recombination of 5.2% was estimated. The three markers segregated together in 67 plants. There were as many recombinants for *Sec-3*–850 as for *Sec-3*–*Sec-1* (Table 3).

The results for the second group are quite similar. The combined segregation of *Sec-1*, the interchange, and

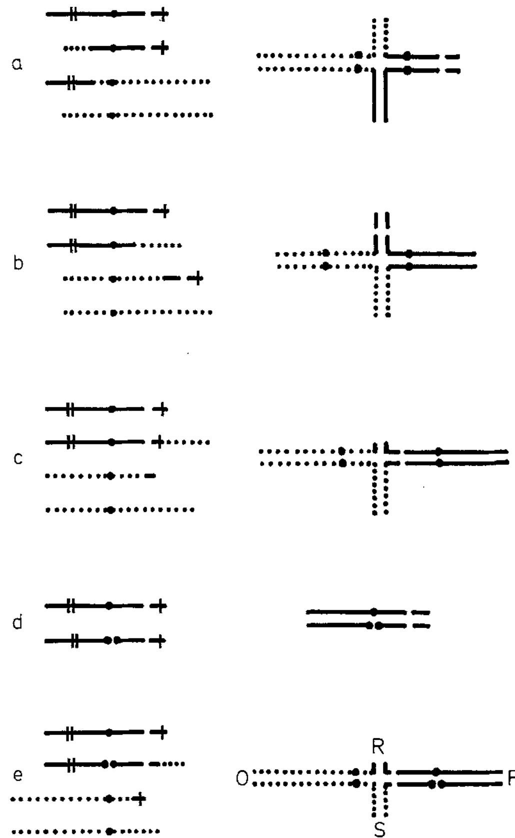

Fig. 1a–e. Diagrams of chromosomes (left) and meiotic pairing configuration (right) of five chromosomal rearrangements in rye. Chromosome *1R* solid, other chromosomes *dotted*. Most probable locations of *Sec-1* (′) and *Sec-3* (″) indicated. Centromeres: large *dots*. NOR: open *segment*. **a** Interchange T273W (*1RL/5RS*). **b** Interchange T306W (*1RS/6RL*). **c** Interchange T850W (*1RS/4RL*). **d** Centromere split Rb1RW (*1R*). **e** Interchanged centromere split Rb1R/248W (*1RL/1RS/6RS*)

Sec-3 in 116 plants is shown in Table 4. Recombination between *Sec-1* and *Sec-3* was 12.9%, between *Sec-1* and T850W it was 1.7%, and between T850W and *Sec-3*, 11.2%. The two groups of families together (Tables 3 and 4) gave: *Sec-1*–*Sec-3*: 14.2%; *Sec-1*–T850W: 2.2%; T850W–*Sec-3*: 12.0%. However, the recombination frequencies do not clearly suggest a physical order for the three loci. Meiotic observations from which an indication can be deduced are discussed below.

The recombination between *Sec-1* and *Sec-3* in a population of 139 plants, including those considered in the joint segregation with the interchange (Table 4) of 9.35%, was much lower than observed by Lawrence and Appels (1986), apparently due to the disturbing effect of the translocation. This was expected especially in the short arm, where the break is situated.

Meiotic metaphase I configuration frequencies were scored in 200 pollen mother cells of one heterozygote from the second group. There were no open-ring quadri-

Table 1. Segregation of interchange T273W (*1RL/5RS*) and *Sec-3* (HMW secalins) in rye; F_1 between T273W and line 129 test-crossed with T273. TT – homozygotes for the rearrangement, NT – heterozygotes

T273W	NT	TT		
Sec-3				
+	33	4	37	
–	3	22	25	
				Recombination:
Total	36	26	62	*Sec-3*/273: 7/62 = 11.3%

Table 2. Segregation of interchange T306W (*1RS/6RL*) and *Sec-1* (40-K gamma secalins); F_1 between T306W and line 129 test crossed with line 129. NT – rearrangement heterozygotes, NN – normal karyotypes

T306W	NT	NN		
Sec-1				
+	37	13	50	
–	12	42	54	
				Recombination:
Total	49	55	104	*Sec-1*/306: 25/104 = 24%

valents, one "figure eight", 159 chain quadrivalents, 28 "frying-pan" quadrivalents, 7 trivalents with univalent, and 5 sets of two bivalents, some of which may have been rings. How many were rings and how many were open bivalents could not be determined, because the bivalents that were derived from the interchange complex were not unequivocally distinguishable from the bivalents that were not derived from the interchange. Application of the formulae of Sybenga (1975) leads to estimates of chiasmate association frequencies for the two non-translocation arms O and P (Fig. 1c) of 1.00 and 0.98, respectively. One interstitial segment, probably that in *1R*, has 0.15 chiasmata. Because of 50% involvement of the four chromatids and 50% recombinant gamete loss due to segregation, 25% of these 15% contribute 3.75% to recombination between the translocation break in the satellite and *Sec-3*. The remaining 8.25% of the 12% recombination observed for this segment should be due to exchange in the segment between the centromere and *Sec-3* in *1RL*. This corresponds reasonably well with the values determined for this distance.

From the meiotic observations it appears that recombination in one of the interchanged segments was (practically) absent, and in about 47% the other (0.95 chiasmate association). The segment translocated to the satellite in *1RS* is many times the size of the segment translocated from the satellite to *4RL*, which includes nothing or little more than the terminal C-band. The first interchanged segment must, therefore, be assumed to have the larger chiasma frequency. If the meiotic observations on the plant studied corresponded with the situ-

Table 3. Segregation of interchange T850W (*1RS/4RL*), *Sec-1* (omega secalins), and *Sec-3* (HMW secalins); F_1 of T850W and line 129 test-crossed with T850W. Families 86634 – 86636. NT and TT as in Tables 1 and 2

T850W		NT	TT		
Sec-3	*Sec-1*				
+	+	27	0	27	
+	–	1	3	4	
–	+	5	0	5	Recombination:
–	–	1	30	31	*Sec-1*/850: 2/67 = 3.5%
					Sec-3/850: 9/67 = 13.4%
Total		34	33	67	*Sec-1*/*Sec-3*: 9/67 = 13.4%

The same families and, in addition, families 86632 and 86633, a total of 134 plants scored only for T850W and *Sec-1*, showed 7 recombinants, giving 5.2% recombination

Table 4. Segregation of interchange T850W (*1RS/4RL*), *Sec-1* (40-K gamma secalins) and *Sec-3* (HMW secalins); F_1 of cross between T850W and line 129 testcrossed with line 129. Families 87362–87366. For NT and NN see Tables 1 and 2

T850W		NT	NN		
Sec-3	*Sec-1*				
+	+	49	1	50	
+	–	1	6	7	
–	+	6	0	6	Recombination:
–	–	0	53	53	*Sec-1*/850: 2/116 = 1.7%
					Sec-3/850: 13/116 = 11.2%
Total		56	60	116	*Sec-1*/*Sec-3*: 13/116 = 11.2%

In the same families, among a total of 139 plants, including those that could not be scored for T850W, 13 recombinants between *Sec-1* and *Sec-3* were observed; recombination 9.4%

Tables 3 and 4 combined:
Sec-1/850: 9/259 = 3.6%
Sec-3/850: 22/183 = 12.0%
Sec-1/*Sec-3*: 22/206 = 10.7%

ation in the heterozygous parent, the *Sec-1* locus could not be in the interchanged segment, which never showed a chiasma. At least $2 \times 0.022 \times 200 = 8.8$ chiasmata would be expected in the 200 cells studied, if the locus was situated in this segment. Consequently, it must be located proximal to the break in the segment of the satellite still attached to *1RS*. All recombination between *Sec-1* and the translocation must then be due to exchange in the interstitial segment, somewhere between the NOR and the breakpoint. This accounts for most of the 3.75% recombination in the interstitial segment expected on the basis of chiasma formation.

The order, then, of the loci used for the calculations of the genetic distances is: T850W–*Sec-1*–*Sec-3*. Two double crossovers ($+-N$ and $-+T$) were found and $(22/183) \times (4/183) \times 83 = 0.48$ were expected, which does not suggest interference. The recombination frequencies adjusted for double crossing-over were *Sec-1*–T850W: 2.2%; *Sec-1*–*Sec-3*: 14.2%; and *Sec-3*–T850W: 12%.

Table 5. Segregation of Robertsonian centromere split Rb1R of genotype 016 and *Sec-3* (HMW secalins); F_1 between Rb1R and line 129 test-crossed with Rb1R. Families 86441 and 86482

Rb1R	NT	TT		
Sec-3				
+	26	3	29	
−	5	19	24	Recombination:
Total	31	22	53	*Sec-3*/Rb1R: 8/53 = 15.1%

Table 6. Segregation of Rb1R genotype 029 and *Sec-3* (HMW secalins); F_1 of Rb1R and line 129 testcrossed with inbred line 029. Families 86642 and 86643

Rb1R	NT	NN		
Sec-3				
+	19	3	22	
−	6	23	39	Recombination:
Total	25	26	51	*Sec-3*/Rb1R: 9/51 = 17.6%

Table 7. Segregation of Rb1R genotype 016 and *Sec-1* (omega secalins); F_1 between Rb1R and line 129 testcrossed with line 129. Families 86442, 86467–86469 and 86472

Rb1R	NT	NN		
Sec-1				
+	50	23	73	
−	21	51	72	Recombination:
Total	71	74	145	*Sec-1*/Rb1R: 44/145 = 30.3%

Table 8. Segregation of Rb1R genotype 029, *Sec-1* (40-K gamma secalins) and *Sec-3* (HMW secalins); F_1 between Rb1R and line 129 testcrossed with line 129. Families 87376 and 87377

Rb1R		NT	NN		
Sec-3	*Sec-1*				
+	+	3	1	4	
+	−	4	0	4	Recombination:
−	+	0	2	2	*Sec-1*/centrom.: 7/19 = 36.8%
−	−	0	9	9	*Sec-3*/centrom.: 1/19 = 5.3%
Total		7	12	19	*Sec-1*/*Sec-3*: 6/19 = 31.6%

The same families and, in addition, the remaining families of 87372–87381 scored only for Rb1R and *Sec-3* (total of 109 plants) showed 10 recombinants: recombination 9.2%
Recombination *Sec-3*/Rb1R from Tables 5, 6, and 8 combined: 27/213 = 12.7%

Rb1R

There were four groups of segregating populations.

(a) Families 86441 and 86482 had the rearrangement of the 016 genotype (HMW-). Only HMW secalins segregated (Table 5). Recombination was 15.0%, rather high for the distance between the centromere and *Sec-3*, taking into account a possible reduction in pairing as a result of the centric split (Fu and Sears 1973).

(b) Families 86642, 86643, with the rearrangement of genotype 029 (Table 6) had a recombination between *Sec-3* and the centromere of 17.6%.

(c) Families 86442, 86467, 86468, 86469, and 86472, with the rearrangement of genotype 016 (Table 7), had a recombination between the centromere and *Sec-1* (omega secalins) of 30.3%.

(d) Families 87372–87381 (10 small populations) with Rb1R of the 029 genotype. Table 8 shows the combined segregation of Rb1R with HMW secalin (*Sec-3*) and 40-K gamma secalin (*Sec-1*) in populations 86376 and 86377. There was one double-crossover. The recombination was: *Sec-1*–*Sec-3:* 42.1%; *Sec-1*–contromere: 36.8%; and centromere–*Sec-3:* 5.3%. The segregation for Rb1R and HMW secalin in all populations pooled is also given in Table 8. The recombination was 9.2%, somewhat lower than in the previous cases.

Rb1R/248W

(a) Families 86638-86640 segregated for Rb1R/248W, *Sec-1* (omega secalins), and *Sec-3* (HMW secalins) (Table 9). The order of the three loci is known and one double-crossover could be recognized. The recombination frequencies adjusted for the double-crossover are: *Sec-1*–*Sec-3:* 8/31 = 25.8%; *Sec-1*–centromere: 19.4%; and centromere–*Sec-3:* 6.5%. The families were small. There were an additional 12 1RS/248W telocentric trisomics, and all were omega secalin$^+$ and HMW secalin$^-$. The implications are discussed below.

(b) Families 87435 and 87437 segregated for Rb1R/248W, HMW secalin (*Sec-3*), and 40-K gamma secalin (*Sec-1*) (Table 10). The recombination was: *Sec-1*–centromere: 22.2%; centromere–*Sec-3:* 0%; and *Sec-1*–*Sec-3:* 22.2%. Recombination among a total of 53 plants, scorable only for HMW and 40-K gamma secalins, was 26.4%. There were four 1RS/248W telocentric trisomics, all 40-K gamma secalin$^+$, and segregating for HMW secalin.

(c) Families 87367–87371 segregated for Rb1R/248W, *Sec-3* (HMW secalin), and *Sec-1* (40-K gamma secalin) (Table 11). No double-crossovers were observed. The segregations were somewhat distorted, with the recombination frequencies being: *Sec-1*–centromere: 21.1%; centromere–*Sec-3:* 1.8%; and *Sec-1*–*Sec-3:* 22.9%. In 100 cells analyzed at first metaphase of meiosis, only one interstitial chiasma was observed. Products of recombination in the interstitial segment are at least 50% lethal. Recombination between *Sec-1* and the centromere must, therefore, have taken place (almost) exclusively in the interchanged segment. The chiasmate association of this segment at metaphase I of meiosis was 38%, correspond-

Table 9. Segregation of translocated Robertsonian split Rb1R/248W, *Sec-1* (omega secalins), and *Sec-3* (HMW secalins); testcrossed with Rb1R/248. Families 86638–86640. NT and TT as in Tables 1 and 2

Rb1R/248		NT	TT		
Sec-3	*Sec-1*				
+	+	0	3	3	
+	–	1	7	8	Recombination:
–	+	17	0	17	*Sec-1*/centrom.: 6/31 = 19.4%
–	–	2	1	3	*Sec-3*/centrom.: 2/31 = 6.5%
Total		20	11	31	*Sec-1/Sec-3:* 6/31 = 19.4%

Among 66 plants of the same families scored only for *Sec-1* and *Sec-3*, 11 recombinants or 16.9% recombination

Table 10. As in Table 9, families 87435 and 87437, *Sec-1* scored for 40-K gamma secalins

Rb1R/248		NT	TT		
Sec-3	*Sec-1*				
+	+	5	0	5	
+	–	13	0	13	Recombination:
–	+	0	8	8	*Sec-1*/centrom.: 6/27 = 22.2%
–	+	0	1	1	*Sec-3*/centrom.: 0/27 = 0%
Total		18	9	27	*Sec-1/Rb1R3:* 6/27 = 22.2%

Same families, 14 recombinants among 53 plants scored only for *Sec-1/Sec-3:* 26.4% recombination

Table 11. Segregation of Rb1R/248, *Sec-1* (40-K gamma secalins) and *Sec-3*, as in Table 9, but testcross with line 129. Families 87367–87371

Rb1R/248		NT	NN		
Sec-3	*Sec-1*				
+	+	38	0	38	
+	–	10	1	11	Recombination:
–	+	1	13	14	*Sec-1*/centrom.: 23/109 = 21.1%
–	–	0	46	46	*Sec-3*/centrom.: 2/109 = 1.8%
Total		49	60	109	*Sec-1/Sec-3:* 25/109 = 22.9%

Total segregation Tables 9, 10, and 11:
Sec-1/centrom.: 35/167 = 21.0%
Sec-3/centrom.: 4/167 = 2.4%
Sec-1/Sec-3: 50/228 = 21.9%

ing to 19% recombination between the breakpoint and the tip of the chromosome. This is equivalent to the 21% recombination assumed for the interchange break and *Sec-1*, and shows that there is practically no recombination distal to *Sec-1*.

There were 16 1RS/248 telocentric trisomics, all with HMW secalin$^-$. This corresponds with the very low recombination frequency in the proximal region of *1RL* in this material. Of the 16 trisomics, 15 were 40-K gamma secalin$^+$ and one was 40-K gamma secalin$^-$, with the latter being a recombinant.

An analysis of meiotic metaphase I behavior of the F_1 heterozygote was necessary to explain the frequent origin of the telocentric trisomics, and an analysis of the trisomics was necessary to identify their type. This shed light on the order of the markers, which was difficult to decide from the segregations, because the recombination frequencies were often very similar for the *Sec-1*–centromere and the *Sec-1*–*Sec-3* intervals. One hundred metaphase I cells of one heterozygous plant of family 87435 of the second group of families were analyzed. Because a plant from a related family that was not systematically analyzed showed a very similar behavior, it is assumed that there was not much variation in meiotic behavior and, therefore, that the segregating parents of the populations studied had essentially the same meiotic characteristics as the progeny plant analyzed.

In the majority of the cells, the complex was composed of two independently orienting parts, a trivalent and a bivalent. This was due to the absence of chiasmata in segment R of Fig. 1e. The consequence was that, when the telocentrics move to posite poles (as occurs in 50% of the cases), one pole receives the non-viable, deficient combination of a normal chromosome *6R* and a telocentric *1RL*. The other pole receives the telocentric translocation chromosome *1RS/248*, the translocated *6RS/248*, and the normal chromosome *1R*. The first two compensate for one normal *6R*, but have extra material from *1R* (equivalent to one arm *1RS*). This combination is viable and results in a "compensating trisomic" when crossed with a normal type. Adjacent orientation of the quinquivalent has the same consequence (de Vries and Sybenga 1989). The trisomics found did indeed show the predicted (de Vries and Sybenga 1989) ratio of quinquivalents and trivalent/bivalent combinations, which identifies the trisomic as a compensating trisomic. If it were a simple telo-tertiary trisomic, the consistent presence of the *Sec-1*$^+$ allele in the trisomic would be difficult to explain. In a compensating trisomic, this linkage is expected. The question now is whether the *Sec-1* locus is in the interstitial segment or in the translocated segment. Both are possible with the observed close linkage of *Sec-1* with the compensating trisomic, because both segments are consistently present in the trisomic. This carries the tip of the satellite of *1RS* in the translocated *6RS/248* and the interstitial segment in the telocentric (Fig. 1e).

Synaptonemal complex studies of heterozygotes of interchange 248 (de Jong et al. 1989) have shown that the break is close to the NOR. At meiotic metaphase I interstitial chiasmata have been observed, but not enough to explain the high recombination frequency between the centromere and *Sec-1*, when this gene is located distal to the NOR but proximal to the interchange breakpoint. This suggests that the locus is in the translocated segment in *6RS/248*. The most frequent origin of the compensating trisomic is due to independent orientation of the

trivalent and the bivalent. In this situation, no recombination between the translocated segment and the normal chromosome is possible. In the less frequent origin due to partial adjacent orientation of the quinquivalent, recombination is possible, and explains the rare recombinant trisomic. The fact that *Sec-3* has recombined only very infrequently in the trisomics is the result of the recombination frequency in the relevant segment always being very low in this material (Tables 9–11). All indications, therefore, point to a location of *Sec-1* in the translocated segment, with the order *Sec-1*–centromere–*Sec-3*. Because there were no double-crossovers, the recombination percentages remain as above.

Discussion

The results are in broad agreement with the observations of earlier authors, but show some differences and present additional information.

The location of *Sec-1* in the satellite of *1R* can be further defined. The conclusion that *Sec-1* is located distally to the breakpoint of interchange 248 but is proximal to that of 805, in combination with the recombination frequencies with the two interchanges, defines its position in the distal portion of the satellite (Table 1 c and e). Synaptonemal complex studies (de Jong et al. 1989) show the breakpoint of 248 to be near but distinctly distal to the NOR. Lawrence and Appels (1986) report 26% recombination between the NOR and *Sec-1*. Our results show recombination of about 20% between 248 and *Sec-1*, about 20% between 248 and the chromosome end, about 2% between *Sec-1* and 850, and 0% between 850 and the chromosome end. Reduction of recombination in the neighborhood of interchange breakpoints in heterozygotes has apparently reduced the genetic distances between *Sec-1* and the two interchanges in the satellite, because recombination between the centromere and *Sec-1* in the absence of interchanges is much higher. It is somewhat surprising, therefore, that a significant, even if small, level of recombination with *Sec-1* was still found very close to the breakpoint of 850. Such asymmetric reduction, however, has been observed before (de Jong et al. 1989).

Genetically, the distance between *Sec-3* and the centromere is small, but this is not necessarily the case in physical terms. It is interesting that the distance from the centromere is, genetically, about the same as that from the breakpoint of interchange 273. There are no direct estimates for the physical distance between the centromere and the breakpoint of 273, but there are a few indications. In 273, the total length of the translocated arm *1RL* is reduced compared to the normal arm *1RL*, and is about the length of the normal *1RS*. The chiasma frequency in the translocated segment from *5RS* in the heterozygote is reduced to about 80% compared with a normal *5RS* (Sybenga 1975). Because *5RS* is a short arm, having chiasma formation like *1RL* concentrated in the distal end, this reduction suggests that the translocated segment is relatively small. A considerable length of *1RL* must, therefore, have remained attached to the centromere of *1R* in the translocated chromosome, and the segment transferred to *5RS* containing *Sec-3* is much smaller than the complete *1RL*. Even so, recombination between *Sec-3* and the breakpoint is about equal to that in the normal chromosome. This shows that the proximal segment of *1RL* is not involved in recombination. Similarly, the *Glu-1* loci of wheat are located distally on the long arms of the group 1 chromosomes (Payne 1987), but recombination with the centromere is only about 9%.

There are more complications in the recombination involving *Sec-3*. Fu and Sears (1973) concluded that the use of telocentric chromosomes leads to underestimation of the genetic distance between a locus and the centromere. Yet the distances between *Sec-1* and *Sec-3* estimated in normal material, in telocentric material, and in interchange heterozygotes do not generally show large variation in the present experiments. This would appear to agree with the conclusion of Dvorak and Appels (1986), who failed to find a reduction in telocentric material and suggested that the reduction observed by Fu and Sears (1973) was due to the special properties of the material used. The genetically long distance between the centromere and *Sec-1* and the absence of recombination in the proximal segment of *1RL* are most likely the main causes of the lack of a telocentric effect. In fact, Fu and Sears (1973) indicated that the effect was greater for loci close to the centromere. In the present material, there may be some reduction in recombination between *Sec-3* and the centromere when normal telocentric chromosomes are used, but it is certainly small.

Different genotypes were involved and much stronger reduction was observed in several families when the translocated telocentrics 248 were used. The translocation, however, is in the other arm. An extra complication is that observations on meiotic metaphase I showed a high chiasma frequency in the long arm, with chiasma positions not restricted to the distal segment. Among a total of 100 cells analyzed, 3 had a univalent telocentric *1RL*. In view of the overall chiasma frequency and position in this arm and in view of previous data, no univalent was expected. The quadrivalent was maloriented, and it may be assumed that the multivalents had undergone irregular prophase development. Of the remaining 97 cells, 7 had a univalent translocated *1RS* and 35 had a quinquivalent, almost exclusively a chain, while 55 had a trivalent and a bivalent. The segment that failed to form a chiasma in the latter combination was almost consistently the small translocated segment of 248.

Table 12. Chiasma location in *1RL* in Rb1R/248, in quinquivalents (V) and trivalent/bivalent combinations (II+III). a – two chiasmata, one median, on terminal or subterminal; b – one median chiasma; c – one subterminal chiasma; d – one terminal chiasma

Position	a	b	c	d	Total
Configuration					
V	7	15	12	1	35
III+II	9	14	29	3	55
Total	16	29	41	4	90

Heterogeneity χ^2: 4.06; (2 *df*); $P=0.131$ (c and d pooled)

Table 13. Summary of the recombination percentages between the two endosperm storage protein gene loci *Sec-1* and *Sec-3* in chromosome *1R* of rye, and five rearrangements involving this chromosome. Between brackets: numbers of recombinants per total in family

Rearrangement	Recombination (%)		
	Sec-1/rearr.	*Sec-3*/rearr.	*Sec-1*/*Sec-3*
T273W (*1R/5R*)	–	11.3 (7/62)	–
T306W (*1R/6R*)	24.0 (25/104)	–	–
T805W (*1R/4R*)	3.6 (9/250)	12.0 (22/183)	10.7 (22/206)
Rb1RW	31.1 (51/164)	12.7 (27/213)	31.6 (9/16)
Rb1R/248W (*1R/1R/6R*)	21.0 (35/167)	2.4 (4/167)	21.9 (50/228)

In Table 12 the frequencies of double chiasmata, one median, one subterminal, and one terminal chiasma in *1RL* for the two configurations are given. There is a slight, and not quite significant, preference for distal location of chiasmata in the trivalent-bivalent combination. Overall, two chiasmata occurred in 16 cells, with one chiasma occasionally rather proximally located, and 29 cells had a single chiasma near the middle of the arm. Terminal chiasmata were quite rare, in contrast with the short arm, where most chiasmata in the longer exchanged segment had the appearance of being terminal. The apparent location of chiasmata at metaphase I may be misleading, especially when they appear to be predominantly (sub)terminally located. When they are seen to be more proximal, the observation is more reliable.

It can be concluded that chiasma location in *1RL* is to a great extent non-distal, and yet the most proximal segment remains free of chiasmata. The plant analyzed cytologically was a progeny of the original plant segregating for the markers, which had died by the time it was realized that an analysis of meiosis was necessary. Although a sister plant showed the same pattern, it is in principle possible that there was a genetic difference conditioning chiasma localization. It should be noted that the translocated arm *1RS*, which has a relatively high interstitial chiasma frequency in normal heterozygotes, had only one interstitial chiasma in the material studied. This points to a similar reduced chiasma frequency in the centromeric region in this arm, not found in the simple Rb1R heterozygote.

A review of the recombination percentages between the two *Sec* loci and the five rearrangements is given in Table 13.

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