Theor Appl Genet (1995) 91: 720–726

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A genetic map of rye chromosome 1R integrating RFLP and cytogenetic loci

Received: 15 October 1994 / Accepted: 7 March 1995

Abstract A genetic map of rye, *Secale cereale* L., chromosome 1R covering 247 cM was constructed utilizing 27 RFLP and four C-band markers, including terminal C-bands. Genetic mapping of C-bands and the centromere, and *in situ* hybridization of three RFLP clones, allowed for the integration of the genetic and cytological maps. Eight contact points between the genetic and cytological maps revealed variation in the recombination distance to cytological distance ratio ranging between 0.25 and 1.95, a 7.8-fold difference. Recombination was found to be highest in the satellite region of 1RS and lowest in the most distal region of 1RL.

Key words *Secale cereale* · RFLP · Cytogenetic mapping · C-band · *In situ* hybridization

Introduction

A genome can be mapped using two basic approaches: physical (including cytogenetic) and genetic mapping (Heslop-Harrison 1991). Genetic mapping orders DNA sequences along the length of the chromosome according to the frequency of recombination that occurs between loci during meiosis. This approach gives the relative genetic distances among loci but does not indicate their relationship to physical distances or positions along the chromosome. Cytological mapping involves the characterization of chromosome landmarks that can be seen under the light microscope. The perspective of the physical architecture of the chromosome is important in understanding genome organization and also provides critical information for map-based cloning strategies (Wicking and Williamson 1991), which require knowledge of the centimorgan (cM)/kilobase (kb) ratio in the area of interest to be effective. Also, chromosome structural features such as heterochromatin and position within the chromosome influence recombination and gene expression.

Investigations relating genetic and cytological distances for many species have shown a non-correspondence between the two types of measurements. Non-correspondence has been found in maize (Phillips 1968), rice, *Oryza sativa* L., (Gustafson and Dillé 1992), and wheat, *Triticum aestivum* L. em Thell. (Dvořák and Appels 1986). A "hot spot" of recombination was observed in the *bz* locus of maize (Dooner et al. 1985), whereas a "cold spot" of recombination was found in the *Tm-2a* region of tomato, *Lycopersicon esculentum* (Ganal et al. 1989). Recombination has been found to be skewed physically toward the ends of chromosomes in several taxa, including the Triticeae. This trend has been reported in barley, *Hordeum vulgare* L., (Linde-Laursen 1979, 1982; Kleinhofs et al. 1988), wheat (Snape et al. 1985; Jampates and Dvořák 1986; Tsujimoto and Noda 1990), and *Drosophila* (Mather 1938). Szauter (1984) found virtually no recombination within the heterochromatin of chromosomes of *Drosophila* females and a smaller crossover per unit cytological length ratio around centromeres and telomeres, with crossover distribution varying in the euchromatin. In *Saccharomyces cerevisiae*, a comparison of recombination to physical distance for chromosome III demonstrated that variation in the cM/kb ratio for different intervals along the chromosome was at least tenfold, with the ratio being lowest close to the centromere and highest midway down each arm (Oliver et al. 1992).

Werner et al. (1992) used a series of sub-arm deletions in wheat group 7 chromosomes to cytologically map loci that had been genetically mapped by restriction fragment length polymorphism (RFLP). They found striking differences between cytological and genetic distances, both in regions possessing and lacking heterochromatin, with a suppression of recombination in the proximal 70% of the chromosomes and high recombination at the ends. Kota et al. (1993) working with chromosome 1B of wheat, Curtis

Communicated by P. L. Pfahler

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and Lukaszewski (1991) working with 1B of *T. turgidum* L. var '*durum*' and *T. dicoccoides* Korn, and Lukaszewski and Curtis (1993) working with B-genome crosses found similar trends between the genetic and cytological maps. The latter authors suggested that the skewing of recombination to chromosome ends may be due to distal regions having more opportunity for the establishment of chiasmata, with more proximal chiasmata being suppressed by chiasma interference.

In rye, *Secale cereale* L., non-correspondence between genetic and cytological distances has been observed (Sybenga et al. 1990) as well as the trend of recombination being skewed to the distal regions of chromosomes (Lawrence and Appels 1986; Gustafson et al. 1990; Baum and Appels 1991; Lukaszewski 1992; Alonso-Blanco et al. 1993). Lukaszewski (1992) mapped the recombination of six C-bands, including the terminal ones, in 1R and found a total genetic length of 1R of 93.7 recombination frequency (RF). Orellana et al. (1993) mapped both terminal C-bands on 1R with *Sec1* (ω- and γ-secalins) and *Sec3* and found a total genetic length of 1R of 122 RF. Alonso-Blanco et al. (1993) mapped four C-bands, including the terminal ones, with several isozyme loci and a translocation breakpoint and found a total genetic length of 1R of 107.9 RF (128.1 cM).

Wang et al. (1991) and Devos et al. (1993) generated RFLP maps of chromosome 1R measuring 106 and 152 cM, respectively. Given that all previous genetic maps of 1R contain rather large gaps (where undetected recombination would be expected) and that the RFLP maps did not have endpoints, it can be deduced that the total genetic length of 1R is considerably greater than 150 cM. At the molecular level, 1R is comprised of approximately one gigabase pair (Gb) DNA (Lukaszewski et al. 1982).

Gustafson and Dillé (1992) demonstrated that physical coverage of even moderately saturated RFLP genetic maps can be quite limited. The mapping of C-bands permits integration of genetic and cytological maps (Linde-Laursen 1979; Lukaszewski and Curtis 1993). The objectives of the present research were to (1) generate a genetic linkage map of rye chromosome 1R integrating cytological markers with RFLP markers, thus allowing a direct comparison of cytological and genetic distances; (2) place ends on the genetic map given that terminal C-bands could be genetically mapped; and (3) estimate the cytological distance involved in the gap present in the RFLP map.

Materials and methods

Mapping population

An F_2 population was created from a cross between UC-90 and E-line ryes. UC-90 (Gustafson et al. 1973) is a dwarf rye line with a standard C-band karyotype (Sybenga 1983). The E-line (Giraldez et al. 1979) karyotype has lost approximately 80% of the 11 telomeric C-bands of rye. The two parents were selected because they are polymorphic for eight C-bands on chromosomes 1R, 6R and 7R, including three on 1R (Fig. 1). Root tips were collected from individual F_2 plants for mitotic C-banding. The plants were then grown in the greenhouse, self-pollinated, and seed was harvested. F_3 plants were grown in the greenhouse and tissue was bulked from a minimum of 16 individuals from each F_2 family, thus reconstructing the original F_2 genotypes. The mapping population consisted of 111 F_2-derived F_3 families.

Fig. 1 C-band pattern of chromosome 1R from hybrids between UC-90 and E-line. The *left* UC-90 and E-line chromosomes are from the same cell, an F_1 between the two parents; similarly for the *middle* and *right* chromosomes. The first band proximal to the terminal band on 1RS is the NOR (indicated by *open pointers*). Three polymorphic C-bands were mapped (indicated by *solid pointers*): the two terminal C-bands (CTer-1RS, CTer-1RL) and the C-band proximal to the NOR in E-line (C1-1RS)

DNA extraction and Southern analysis

Young leaf tissue was cut and kept on ice until the samples were quick frozen in liquid nitrogen. Samples were then freeze-dried. Tissue was ground to a fine powder using a Tecator Cyclotec mill and stored at –20°C. Genomic DNA was isolated essentially according to Gardiner et al. (1993) except that two phenol extractions were performed. Genomic DNA was digested with one of four restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, or *Hin*dIII) according to manufacturers' instructions except that reactions were run with 2.5 m*M* spermidine. Gels containing 0.7% agarose were run in 0.5×TBE (0.045 *M* TRIS-borate, 0.001 *M* EDTA) at 6–15 mA. Southern transfer, oligolabeling, hybridizations and autoradiography were carried out essentially as in Gardiner et al. (1993) except for the following modifications: MagnaGraph, MagnaCharge (MSI) and Hybond-N+ (Amersham) membranes were used. Clones were amplified by plasmid mini-prep (Gardiner et al. 1993) or by polymerase chain reaction (PCR) from *E. coli* colonies (Güssow and Clackson 1989). Two 5 min post-hybridization room temperature washes were done in 2×SSC (1×SSC: 150 m*M* NaCl, 15 m*M* Na citrate), 0.5% SDS, and three 30 min and one 60 min 65°C washes were done in 0.1×SSC, 0.1% SDS.

RFLP probes

A *Pst*I genomic digest of 'Imperial' rye was size-fractionated by ultracentrifugation in a 10–40% sucrose gradient, and fractions containing approximately 0.4–2.5-kb DNA fragments were ligated into pUC19 by standard methods (Sambrook et al. 1989). *Pst*I was used to generate the library because it is a methylation-sensitive endonuclease and minimizes the number of repetitive sequences cloned (Helentjaris 1987). These clones were given the designation pSCG# for *Secale cereale* genomic. Clones were also generously supplied by other laboratories (see Table 1). pScR4.T1, detecting the nucleolus organizer region (*NOR*), was mapped using *Taq*I-digested genomic DNA.

Cytological mapping protocols

The giemsa C-banding technique of Lukaszewski and Gustafson (1983) was used. For each F_2 individual, the karyotype for each C-band was determined from at least five clear cells. *In situ* hybridization was carried out using the method of Gustafson et al. (1990) on 'Blanco' rye, which has the same C-band pattern as UC-90 (see Fig. 2). Measurements were taken on between 9 and 16 different chromatids, and then averaged, for each probe. C-band locus nomenclature was adapted from Baum and Appels (1991) and Lukaszewski (1992). The prefix C was used to facilitate recognition of C-band

Table 1 DNA clones used as markers on chromosome 1R

1) Known function clones

Locus	Clone	Function	Reference
XNor	pScR4.T1	Rye ribosomal spacer DNA	Appels et al. 1986
XSec3 (*XGlu*)	pTag1290	Wheat HMW glutenin	Thompson et al. 1983
XPgk1	P7	Wheat chloroplast phosphoglycerate kinase	Longstaff et al. 1989
XAdh	p3NTR	3′ Untranslated wheat alcohol dehydrogenase	Mitchell et al. 1989
XAdpg2	pSh2.25	ADP glucose pyrophosphorylase	Devos et al. 1993

2) Anonymous clones

Code	Laboratory	Type	Reference
ABC	A. Kleinhofs, NABGMP[a]	Barley seedling cDNA	Kleinhofs et al. 1993
BCD	M. Sorrells, Cornell University	Barley cDNA	Heun et al. 1991
CDO	M. Sorrells, Cornell University	Oat cDNA	Heun et al. 1991
PSR	M. Gale, IPSR, UK	Wheat gDNA	Devos et al. 1993
SCG	J. P. Gustafson, University of Missouri	Rye gDNA	This paper
WG	M. Sorrells, Cornell University	Wheat gDNA	Heun et al. 1991

[a] North American Barley Genome Mapping Project

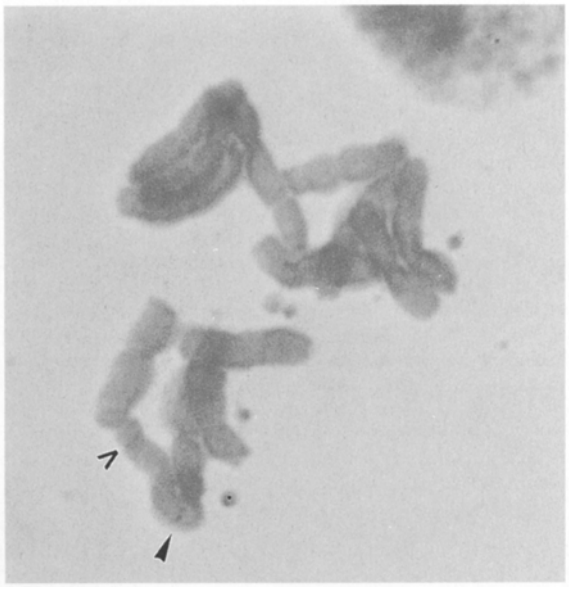

Fig. 2 *In situ* hybridization of wheat genomic clone WG241, approximately 500 bp in size, to the long arm of chromosome 1R. Both chromatids are labeled. *Open pointer* secondary constriction on 1RS, *solid pointer in situ* hybridization site

loci among other types of loci on a genetic map. The correspondence of common bands with Lukaszewski (1992) was as follows: *CTer-1RS≡S3*; *NOR≡S2*; *C1-1RS≡S1*; *CTer-1RL≡L3*. The cytological location of *Sec3* was taken from Gustafson et al. (1990).

Genetic map construction

Multipoint maximum likelihood linkage analysis was performed using Mapmaker version 2.0 (Lander et al. 1987) supplied by DuPont, on a Macintosh IIcx computer. Mapping analysis followed the strategy outlined in the manual. The defaults, minimum LOD=3.0 and maximum theta=0.40, were used. The *Group* command was used to establish an initial potential linkage group, using only codominant markers. Using the *Three-Point* command, a three-point linkage group was established. The *LOD Table* command was then used to select a core of seven markers that were not too closely linked but possessed high LOD scores. A multipoint maximum likelihood core map was then generated using the *Compare* command. Markers which were associated with the linkage group using the *Group* command were then added sequentially to the core map using the *Try* command. After each *Try*, the map was checked with the *Ripple* command to confirm the best order with the new marker. After all codominant markers had been evaluated, dominant (presence/absence) markers were analyzed. Finally, the map was run through the *Drop Marker* command to check for markers that were contributing an inordinate cM distance or LOD to the map, and the *Show Raw* data command to check for double crossovers. Autoradiographs were double-checked for all markers indicated by the *Drop Marker* command, and all double crossovers. RF was converted to cM using the Kosambi (1944) function.

Results and discussion

We screened 385 clones and observed the following levels of polymorphism for the various classes of clones: rye genomic DNA (gDNA) 46%; wheat cDNA 65%; wheat gDNA 60%; barley cDNA 48%; oat cDNA 30%. The level of polymorphism revealed by the four restriction enzymes used was as follows: *Dra*I 30%; *Eco*RV 26%; *Eco*RI 23%; *Hin*dIII 19%. No polymorphism was detected between the parents for *Sec1* and the 5S ribosomal genes on 1RS using the four restriction enzymes mentioned above and an additional eight enzymes: *Bam*HI, *Bgl*II, *Hae*III, *Rsa*I, *Sal*I, *Taq*I, *Xba*I, and *Xho*I.

A genetic map of rye chromosome 1R integrating RFLP markers, C-bands, and the centromere was generated (Fig. 3). The map spans 247 cM, from terminal C-band to terminal C-band and includes 30 loci. The centromere was placed within a 9-cM interval by clones previously tested against the 'Chinese Spring' nullisomic-tetrasomic (Sears 1954) and ditelosomic (Sears and Sears 1978) lines (Devos et al. 1993; M. Sorrells, personal communication). Three gaps ≥30 cM were present on the genetic map. All the marker groups were confirmed to be present on 1R by C-banding or by cytogenetic stocks (as above, with the centromere). Genetic mapping of the four C-bands and the cen-

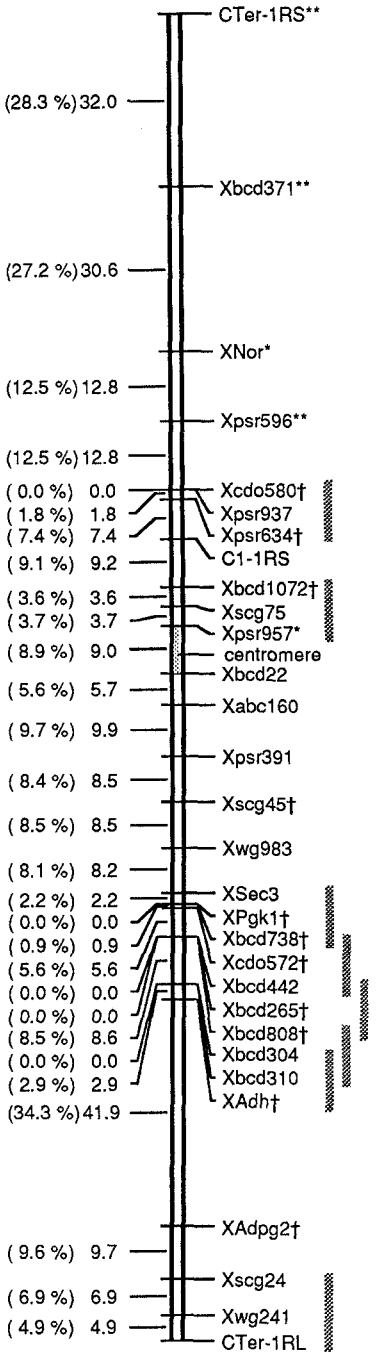

Fig. 3 Genetic map of rye chromosome 1R. The prefix *X* indicates a cloned DNA marker (See Table 1 for details); *C* indicates a C-band. Markers exhibiting distorted segregation are indicated by * for $P=0.05$ and ** for $P=0.01$. All markers were inherited codominantly, except for those indicated by †, which were scored as dominant. Distances are in cM (Kosambi function) with the recombination frequency in *parentheses*. Marker orders with a LOD score of less than 2.5 are indicated by a *vertical bar*. The centromere was placed within a 9-cM interval

tromere, as well as *in situ* hybridization of three genetically mapped RFLP clones, permitted the integration of the genetic and cytological maps. Of the 30 loci five showed segregation distortion (Fig. 3). All of these were skewed in favor of the E-line allele. Several clusters of markers had orders with LOD scores <2.5 (i.e., best order being 316 times more probable than the second-best order). These regions involved either dominantly inherited markers which are less informative than codominant markers, or markers that perfectly cosegregated, except for the three markers on the end of the long arm of 1R (1RL).

Previous linkage analysis in rye has been reviewed by Baum and Appels (1991) and Melz et al. (1992). Of particular relevance to this project was the rye RFLP map created by Devos et al. (1993) as there were eight markers in common between the two 1R maps. Marker order was in agreement between our map and that of Devos et al. (1993), except that the interval *XNor–Xpsr634* was inverted. In this interval, we detected 1.8 cM between *Xpsr937* and *Xpsr634*, whereas the Devos et al. (1993) map showed absolute linkage. Also, all of the markers in this interval in the Devos et al. (1993) map were ordered with LOD scores <2.5. On our map the sequence *Xcdo580–Xpsr937– Xpsr634* was ordered with a LOD score <2.5. Furthermore, in Devos et al. (1993), *Xpsr596* was mapped as a dominant marker, while on the present map *Xcdo580* and *Xpsr634* were mapped as dominant. Given the higher LOD scores for ordering *XNor* and *Xpsr596* in this report, the NOR is probably distal in the *XNor–Xpsr634* block. It is worth noting that Devos et al. (1993) also detected the large gap on 1RL in *XAdh–XAdpg2*. To obtain a good comparison of recombination detected for intervals common between these two mapping populations, common intervals in 7R (Wanous et al. 1995) were also included in a comparison, which showed that the present map distances (cM) were on average greater than those for the Devos et al. (1993) map by a factor of 2.2.

Lukaszewski (1992) mapped the four C-bands included in the present study. The order of the markers is consistent between the two maps. However, the RF observed over the length of the maps was 2.5 times greater for our map. This may be accounted for to some extent by the large gaps present in the Lukaszewski map, where there may have been undetected recombination.

Considerable variation has been reported for the same genetic intervals estimated in different studies in rye (Melz et al. 1992). These differences may be attributed to environmental factors such as temperature or genotypic differences between parents used in the crosses (Fatmi et al. 1993; Tulsieram et al. 1992). These factors may explain the genetic distance differences found between the present study and those of Devos et al. (1993) and Lukaszewski (1992). Rogowsky et al. (1993) placed DNA markers into segments of 1R using wheat-rye recombinants. The positions of common markers in the present map were all consistent with their map.

The barley 1H map generated by Heun et al. (1991) has four markers in common with the long arm of the present 1R map. The order of the markers was the same in the two species: centromere ←*Xwg983–Xbcd265–Xbcd304–Xwg241*→ telomere. The cM distances were 37.8, 0, 30.5 in barley and 16.9, 8.6, 61.4 in rye, respectively. Although the markers were colinear, the pattern of recombination between the common loci was very differ-

Table 2 Cytological measurements of C-band and *in situ* hybridization positions

1. C-bands. Values are fraction length of the entire chromosome excluding the telomeric C-bands	
CTer-1RS – NOR	0.13 ± 0.01
NOR – C1-1RS	0.10 ± 0.00
C1-1RS – centromere	0.17 ± 0.01
Centromere – *CTer-1RL*	0.59 ± 0.01
2. *In situ* hybridization sites. Values are fraction length of 1RL from the centromere	
Sec3	0.43 ± 0.06
Adpg2	0.73 ± 0.02
WG241	0.86 ± 0.02

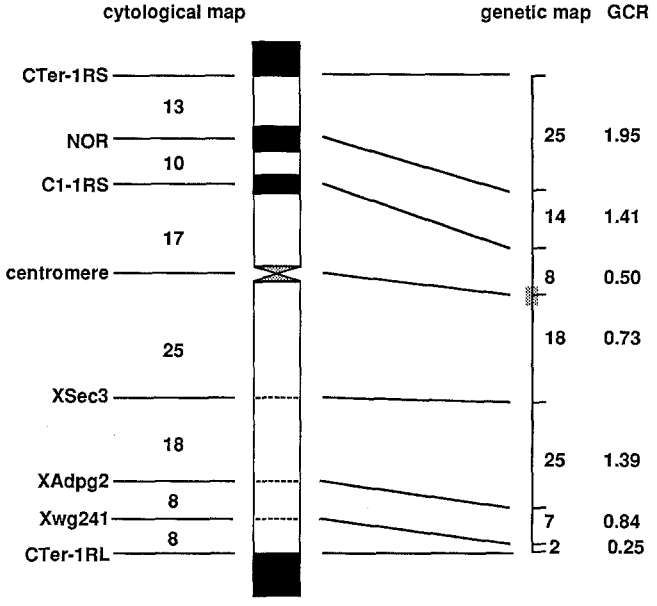

Fig. 4 Comparison of the cytological and genetic maps of chromosome 1R. Both scales are drawn as percentages of the total distance. The centromere is located within a 9-cM interval on the genetic map, which corresponds to 3.6% of the length of the genetic map. *Black bands* represent C-bands; *dotted lines* represent cloned DNA *in situ* hybridization sites. *GCR* the percent genetic length to percent cytological length ratio

ent. Wang et al. (1992) found a similar trend comparing intervals on 1R and 1H. *Xbcd265–Xbcd304* was separated by 8.6 cM in rye but was absolutely linked in barley. The region including the 41.9-cM 1RL gap (a total of 61.4 cM), *Xbcd304–Xwg241,* was found to be smaller in barley (30.5 cM). Conversely, the *Xbcd265–Xwg983* 37.8-cM gap in 1H was smaller (16.9 cM) and contained additional markers in rye. One gap was conserved between the two species but was smaller in barley, and one gap detected in barley was moderately saturated in rye. Gaps of 30 cM and 43 cM were also found distal to an *Adh* locus on maps of 1DL in *Triticum tauschii* (Lagudah et al. 1991). The observation that a recombinational gap was detected with different rye genotypes, in barley and in *T. tauschii,* suggests that there may be conserved gaps within the Triticeae. These gaps may be due to regions that promote high recombination or are recalcitrant to DNA clone recovery.

There were eight points in common between the cytological (Table 2) and genetic maps: four C-bands, three DNA clones placed by *in situ* hybridization, and the centromere (Fig. 4). This created seven regions within which the relationship between cytological and genetic distance could be evaluated. The interval *CTer-1RS–NOR* comprises the satellite in rye. This region had a percent genetic length/percent cytological length ratio (GCR) of 1.95. This was the highest of all the regions. *NOR–C1-1RS* had a GCR of 1.41, *C1-1RS*–centromere had a GCR of 0.50 (assuming the centromere is centered between *Xpsr957* and *Xbcd22*); centromere–*XSec3* had a GCR of 0.73 (same assumption as above); *XSec3–XAdpg2* had a GCR of 1.39; *XAdpg2–Xwg241* had a GCR of 0.84; and *Xwg241–CTer-1RL* had a GCR of 0.25.

The finding that the two regions surrounding the centromere had GCRs of less than 1 was consistent with previous reports of reduced recombination around the centromere in the Triticeae. The GCRs of 1.95 in the satellite and 1.41 in *XSec3–XAdpg2* were in agreement with previous reports showing high recombination in these regions. The two most distal regions on 1RL showed a decreasing GCR moving toward the telomere. *Xwg241–CTer-1RL* comprised the terminal 8% of 1R cytologically and had a GCR of 0.25, the lowest recombination rate on the map. The section of Lukaszewski's (1992) map that involved the terminal 1% of 1R cytologically had a GCR of 3.09. There is not a readily apparent explanation for the dissonance of these results. One possibility is that underestimation of the total genetic length of 1R in the Lukaszewski map due to the use of too few markers led to the inflation of calculated GCR for the terminal 1RL interval. Another possibility is the presence of variation in the distribution of recombination between the two populations, although one parent (E-line) was common in both studies. The recombination level measured in each cytological region was an average over that region and undoubtedly was comprised of components with varying recombination rates. Thus, the distal 8% which overall manifested a GCR of 0.25 may, in fact, have included a most distal 1% with a GCR of 3.09.

The cytological regions examined had a range in GCR from 0.25 to 1.95, a 7.8-fold variation. This is a lower limit of recombination variation within 1R considering that comparisons of smaller regions would likely show greater differences. Variation in GCR along the chromosome can be explained as variation in recombination per unit base pair length or as differences in condensation and chromosome packaging of the DNA molecule. Alonso-Blanco et al. (1993) found that cytological distances on 1RS determined from synaptonemal complexes in pachynema were directly proportional to corresponding intervals in mitotic chromosomes. Thus, differences between cytological distances in mitosis (the stage at which C-bands and *in situ* hybridization sites were measured) and genetic distances do not seem to be attributable to differences in chromosome organization in mitosis versus meiosis when the chromosomes are involved in recombination.

Moore et al. (1993a), studying wheat-rye recombinant chromosomes, found a higher proportion of larger *Not*I and *Mlu*I fragments in the pericentromeric regions and smaller fragments in the distal and subtelomeric regions. This suggested that there is a higher density of unmethylated *Not*I and *Mlu*I sites, which correspond to gene-coding regions, in the distal and subtelomeric regions than in the centric regions. It has also been reported that the centromeric regions of barley and rye chromosomes are more highly condensed than terminal regions, containing more than twice the DNA concentration (Anamthawat-Jónsson and Heslop-Harrison 1990). These observations support a general model of cereal chromosome organization with highly condensed CpG- and CpXpG-methylated repeated sequences in the centric regions and less methylated gene-rich regions toward the termini (Moore et al. 1993b). The results of the present study fit this general model except for the terminal 16% of 1RL. Specific chromosome regions may vary from the general model due to recombinational "cold" and "hot" spots.

Considering that centric regions are more condensed and that they have a low level of recombination, as demonstrated in this and previous studies, the centric regions must have a very low rate of recombination per DNA base pair length relative to the rest of the chromosome. The trend that genes are concentrated in the distal regions, combined with GCR information for specific intervals, will be important for evaluating the feasibility of map-based cloning approaches for genes of interest (Moore et al. 1993b).

The range in recombination rate for different regions of the chromosome has important implications for plant breeding. It may be difficult or impossible to create new combinations of alleles at loci from regions of very low recombination. Conversely, many new allelic combinations would be expected to be possible with genes in high recombination regions.

Acknowledgements Our thanks are extended to the individuals mentioned in Table 1 who provided clones from species other than rye, in particular, M. Gale and M. Sorrells; to K. Houchins for helping with the rye clone screening and mapping hybridizations. K. Houchins, C. L. McIntyre, E. Butler, and J. Gardiner constructed the rye genomic library. K. Ross C-banded the UC-90 X E-line F_1. We thank Jiamin Chen for help with the *in situ* technique. M. K. W. was supported by the Missouri Food for the 21st Century program.

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