Physical and Genetic Mapping in the Grasses *Lolium perenne* and *Festuca pratensis*

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ABSTRACT

A single chromosome of the grass species *Festuca pratensis* has been introgressed into *Lolium perenne* to produce a diploid monosomic substitution line ($2n = 2x = 14$). In this line recombination occurs throughout the length of the *F. pratensis/L. perenne* bivalent. The *F. pratensis* chromosome and recombinants between it and its *L. perenne* homeologue can be visualized using genomic *in situ* hybridization (GISH). GISH junctions represent the physical locations of sites of recombination, enabling a range of recombinant chromosomes to be used for physical mapping of the introgressed *F. pratensis* chromosome. The physical map, in conjunction with a genetic map composed of 104 *F. pratensis*-specific amplified fragment length polymorphisms (AFLPs), demonstrated: (1) the first large-scale analysis of the physical distribution of AFLPs; (2) variation in the relationship between genetic and physical distance from one part of the *F. pratensis* chromosome to another (*e.g.*, variation was observed between and within chromosome arms); (3) that nucleolar organizer regions (NORs) and centromeres greatly reduce recombination; (4) that coding sequences are present close to the centromere and NORs in areas of low recombination in plant species with large genomes; and (5) apparent complete synteny between the *F. pratensis* chromosome and rice chromosome 1.

THERE is considerable evidence that there is not a consistent relationship between genetic distance in centimorgans and physical distance in base pairs and that there is variation in this relationship from one part of the genome to another (*e.g.*, Gustafson and Dillé 1992; Werner *et al.* 1992; Hohmann *et al.* 1994, 1995; Chen and Gustafson 1995; Delaney *et al.* 1995; Mickelson-Young *et al.* 1995; Gill *et al.* 1996a,b; Künzel *et al.* 2000). Genetically close markers may actually be far apart in terms of base pairs (or vice versa) due to differences in the frequency of recombination along the length of a chromosome. When considering the average length of DNA per unit of recombination, different segments of a chromosome should therefore be considered independently. For chromosome 4 of Arabidopsis, the base pair to centimorgan ratio varied from 30 to 550 kb per cM (Schmidt *et al.* 1995). In rice 1 cM is on average equal to 240 kb, although this figure actually varies from 120 to 1000 kb per cM (Kurata *et al.* 1994). In wheat the variation is even more extreme, with 1 cM equal to 118 kb in regions of high recombination but 22,000 kb in regions of low recombination (Gill *et al.* 1996a,b). Regions corresponding to centromeres, and even some telomeres in tomato and potato, show a 10-fold decrease in recombination compared to other regions in the genome (Tanksley *et al.* 1992). Reduced recombination frequency in pericentric regions is also seen in many species including the grasses, *e.g.*, wheat (Dvořák and Chen 1984; Snape *et al.* 1985; Curtis and Lukaszewski 1991; Gill *et al.* 1993, 1996a,b; for review, see Gill and Gill 1994), barley (Leitch and Heslop-Harrison 1993; Pedersen *et al.* 1995; Künzel *et al.* 2000), rye (for review, see Heslop-Harrison 1991; Wang *et al.* 1992), and Lolium (Hayward *et al.* 1998; Bert *et al.* 1999). Nucleolar organizer regions (NORs) may also cause a reduction in the frequency of crossing over (*e.g.*, *Allium schoenophrasum*; J. S. Parker, personal communication). Recombination hot spots also occur (Endo and Gill 1996; Künzel *et al.* 2000; Weng *et al.* 2000).

In this article we describe the physical mapping of a *Festuca pratensis* (meadow fescue $2n = 2x = 14$) chromosome in the progeny of a *Lolium perenne* (perennial ryegrass $2n = 2x = 14$)/*F. pratensis* monosomic substitution (*i.e.*, 13 *L. perenne* chromosomes + 1 *F. pratensis* chromosome). *L. perenne/F. pratensis* monosomic substitutions are unusual because although the *F. pratensis* chromosome and its *L. perenne* homeologue recombine at high frequency they can be distinguished using genomic *in situ* hybridization (GISH). GISH analysis of the *L. perenne/F. pratensis* recombinant chromosomes in progeny derived from the *L. perenne/F. pratensis* monosomic substitution has allowed the determination of the physical position of crossover events between the *L. perenne/F. pra-*

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tensis homeologues. These cytological observations have been combined with data based on dense amplified fragment length polymorphism (AFLP) marker genotypes of the recombinant chromosomes of the same individuals (King *et al.* 2002, accompanying article) to determine the relationship between physical and genetic distance.

MATERIALS AND METHODS

The 14-chromosome *L. perenne/F. pratensis* monosomic substitution plant (backcross individual BC_1 57, which carried a NOR in one arm of the Festuca chromosome) was isolated from the progeny of a cross between a triploid Lolium/Lolium/Festuca hybrid (male parent) and diploid *L. perenne*, c.v. Liprio (female parent; King *et al.* 1998). BC_1 57 was backcrossed (as the male parent) to the same diploid *L. perenne* genotype (female parent) to produce a BC_2 mapping population. A total of 148 of these were randomly chosen and mapped for AFLPs (King *et al.* 2002, accompanying article).

***In situ* hybridization:** GISH analysis using *F. pratensis* genomic DNA as probe and fluorescent *in situ* hybridization (FISH) using 18S-26S rDNA (pTa71; Gerlach and Bedbrook 1979) on mitotic root tip preparations were performed as described by King *et al.* (1998) and Thomas *et al.* (1996), respectively. Slides were analyzed using a Leica DM/RB epifluorescence microscope with filter blocks for 4′,6-diamidino-2-phenylindole (DAPI), fluorescein, and rhodamine. Photographs were taken with a Nikon U-III multipoint sensor system using Fujichrome Sensia II 400 film.

A genetic linkage map of the *F. pratensis* chromosome in the monosomic substitution line BC_1 57, generated using 50 *Eco*RI/*Tru*91 and 54 *Hin*dIII/*Tru*91 *F. pratensis*-specific AFLPs (King *et al.* 2002, accompanying article), was used to select plants from the BC_2 mapping population for physical mapping. These plants were chosen because they showed a relatively even spread of recombination points along the chromosome and, where possible, recombination points on either side of the centromere and NOR.

Measurements taken of the recombinant chromosomes from mitotic root tip preparations in the BC_2 plants were: (1) total length of chromosome, (2) distance of recombination site or sites from both telomeres, and (3) position of NOR site measured from the edge of the NOR nearest to the telomere. Measurements of *L. perenne/F. pratensis* recombinant chromosomes were taken from enlarged projections of at least 10 separate chromosomes for each of the BC_2 plants used for physical mapping.

Chromosome expansion factor: One objective of this work was to express the size of the *F. pratensis* chromosome segments as a percentage of the whole recombinant chromosome, so enabling a comparison of the sizes of the segments to be made. However, a comparison of the size of the *F. pratensis* chromosome segments in different genotypes is valid only if the total length of the recombinant chromosomes in each genotype is the same. The size of the *F. pratensis* genome is 8.9 pg (Bennett *et al.* 1982) while that of the *L. perenne* genome is 8.3 pg (Hutchinson *et al.* 1979), so the *F. pratensis* genome is 7% larger than the *L. perenne* genome; *i.e.*, the larger the *F. pratensis* chromosome segment the larger the recombinant chromosome.

To examine how expansion of the recombined chromosomes was affected by differences in the genome size of *F. pratensis* and *L. perenne*, different arms were compared. The Festuca NOR arm was compared to the Lolium NOR arm and the Festuca non-NOR arm was compared to the Lolium non-NOR arm. The comparisons were made by taking 100 measurements of each of the different arms. BC_2 plants 18, 11, 3/26, 3, 3/10, and 17 were used to obtain the measurements of the Lolium NOR arm (L_l) while BC_2 plants 92, 36, and 6 were used to obtain the measurements of the Festuca NOR arm (L_f). Similarly, measurements of the Lolium non-NOR arm were obtained from BC_2 plants 2/3, 3/23, 19, 99, and 3/2 and the Festuca non-NOR arm from BC_2 56 (see Figures 1 and 2). The average lengths of the NOR and non-NOR arms of the Lolium and Festuca chromosomes were determined from these and the Festuca expansion factors for each arm were estimated as $e = (L_f/L_l) - 1$.

The expansion factor was applied to the measurements of the Festuca segments and to the measurements of the total lengths of the chromosomes in slightly different ways depending on the size of the Festuca segment. For those Festuca segments that were not large enough to include the Festuca centromere the length of the Festuca segment was decreased in size by the expansion factor. For those Festuca segments that were large enough to include the Festuca centromere the Lolium portion of the chromosome was expanded by the expansion factor. The expansion factor was applied in this way because the expansion of the recombinant chromosome was not the same in the two arms.

Measurements taken on the Festuca chromosome in BC_1 57 were: (1) total length of the chromosome, (2) position of the centromere from both telomeres, and (3) position of both edges of the NOR from each telomere. Measurements of the Festuca chromosome in BC_1 57 were made in 10 separate cells. Because these measurements were taken on a complete chromosome (*i.e.*, not recombined), the measurements were converted to percentages and averaged. For simplicity of description we consider the complete, scaled, physical length of the chromosome as 100 physical units (pu).

Restriction fragment length polymorphism: Restriction fragment length polymorphism (RFLP) analysis was carried out using ECL reagents (Amersham, Arlington Heights, IL) with digested genomic DNA transferred by Southern blotting onto positively charged nylon membrane (Amersham). Thirty-seven Cornell anchor probes (Van Deynze *et al.* 1998) located on different Triticeae linkage groups were used to screen *F. pratensis* (Bf 1183), *L. perenne* c.v. Meltra, *L. perenne* c.v. Liprio, the Lolium/Lolium/Festuca triploid hybrid, and BC_1 57 to find markers specific to the Festuca chromosome in BC_1 57. Markers giving Festuca-specific polymorphisms were then used to screen the 16 BC_2 plants used for the physical mapping as described earlier.

Southern analysis of excised AFLP bands: *F. pratensis*-specific AFLP bands were excised from silver-stained polyacrylamide gels (Cho *et al.* 1996) and placed in a 200-μl microcentrifuge tube. The excised bands were reamplified using the relevant primer pair in a PCR reaction. These amplified AFLP products were electrophoresed on an agarose gel and Southern blotted onto Hybond N+ nylon membrane (Amersham). The Southern blots were hybridized with digoxygenin (DIG; Roche, Indianapolis)-labeled total *F. pratensis* genomic DNA overnight. The blots were washed at high stringency and DIG-labeled hybrids detected using CSPD (Roche), *i.e.*, chemiluminescence, and X-ray film. The AFLP bands were then scored, according to the degree of cross-hybridization, as high-, medium-, or low-copy sequences.

RESULTS

Introgressed Festuca segments studied using GISH: The 16 BC_2 plants used for the physical mapping involved single crossovers (with the exception of BC_2 83),

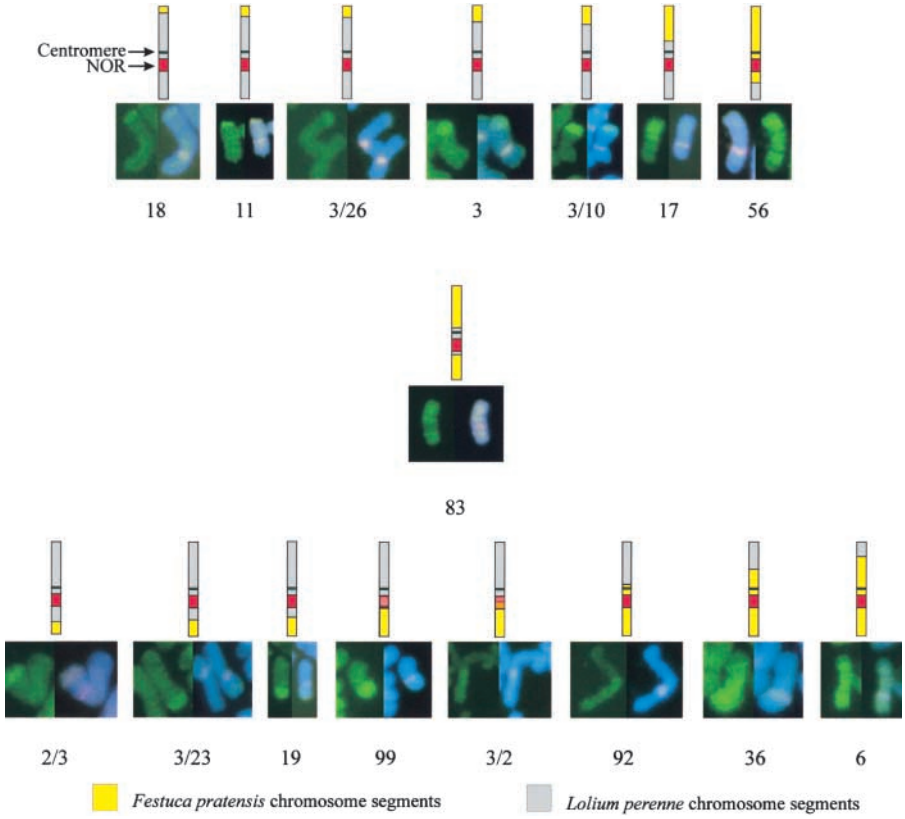


FIGURE 1.—Photographs of the 16 recombinant chromosomes analyzed by GISH and FISH. Genotypes 18, 11, 3/26, 3, 3/10, 17, and 56 form the recombinant series with the Festuca segment increasing in size from the telomere of the non-NOR arm. Genotypes 2/3, 3/23, 19, 99, 3/2, 92, 36, and 6 form the recombinant series with the Festuca segment increasing in size from the telomere of the NOR arm. Genotype 83 is the double-recombinant chromosome with a Festuca segment at both ends. The left side of each pair shows the Festuca segment in bright green after GISH with Festuca total genomic DNA as probe. The right side of each pair shows the recombinant chromosome with DAPI counterstaining and the NOR highlighted after FISH with pTa71 as probe. The Festuca segment appears green or a brighter blue than the Lolium segment.

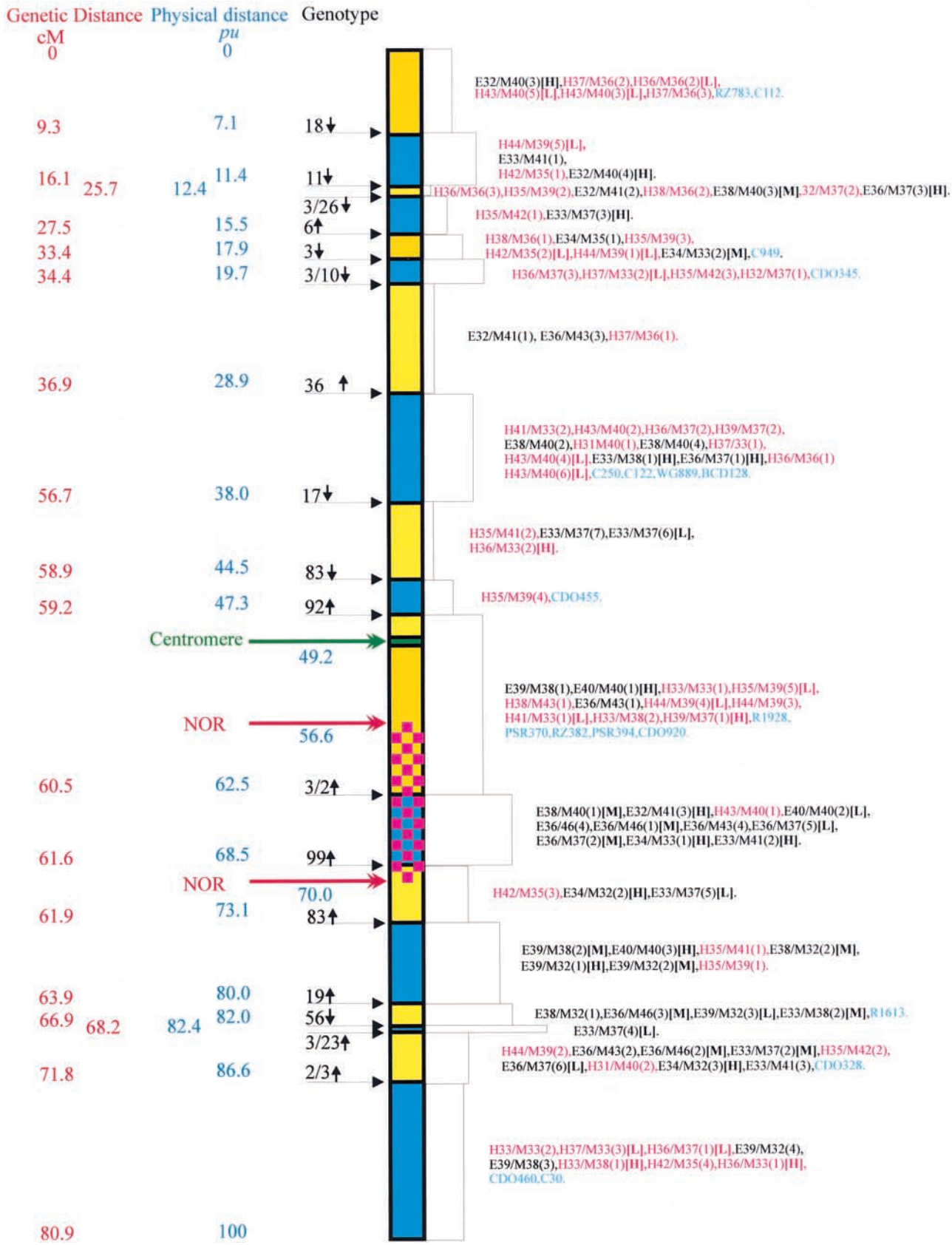
carried segments of a range of sizes (Figure 1), and had from 6 (BC_2 18) to 86 (BC_2 56) Festuca-specific AFLP markers. All of the Festuca segments observed extended from one or other of the telomeres. Recombinant chromosomes carrying interstitial chromosome segments were not examined, although they were available in other genotypes. Thus two series of Festuca segments were looked at using GISH: The first series increased in size from the telomere of the chromosome arm without the NOR, while the second series increased in size from the telomere of the chromosome arm carrying the NOR (Figure 2). This resulted in the Festuca chromosome being split into 18 segments [BC_2 83 contained 2 Festuca segments (Figures 1 and 2) that could be individually measured and mapped].

The expansion of the Festuca chromosome compared to the Lolium chromosome was found to be uneven along the length of the chromosome. Therefore, two separate scaling factors were calculated and applied: 0.2 for the arm containing the NOR and 0.3 for the arm without the NOR.

The physical sites of recombination appeared to occur along the whole length of the chromosome including regions close to the centromere (Figure 2) and within the NOR (Figure 2). Despite the overall good spread of sites there are two gaps, both slightly >10 pu of the total physical length of the chromosome, in which no recombination has occurred among the 148 BC_2 plants of the mapping family. The first of these gaps is located between the site represented by BC_2 92 at 47.3 pu and the site represented by BC_2 3/2 at 62.5 pu (physical distances are from the telomere of the arm without the NOR unless otherwise stated). Genetic mapping of the BC_2 plants (KING *et al.* 2002, accompanying article) in the mapping population showed that there were no plants with a recombination site within this region, which contains both the centromere and a large portion of the NOR. The second of the two gaps in the recombination sites occurred from the site represented by BC_2 2/3 at 86.6 pu to the telomere of the chromosome arm containing the NOR.

Physical distribution of the AFLP markers: The physical distribution of the AFLP markers is displayed in Figure 3. The AFLP markers do not show the same distribution patterns as in the genetic map (KING *et al.* 2002, accompanying article) but show a tendency to cluster. The first 47 pu of the chromosome carries only 34% of the *Eco*RI/*Tru*91 markers, whereas the next 40 pu, *i.e.*, 47–87 pu, carries 62%. The latter coincides with the NOR site that is located between 56.6 and 70 pu along the Festuca chromosome. The remaining 13 pu of the chromosome contained only two (4%) of the *Eco*RI/*Tru*91 AFLP markers.

The *Hin*dIII/*Tru*91 AFLP markers show a more even distribution with peaks between 30–40 pu and 50–60 pu of the chromosome. The two peaks in *Hin*dIII/*Tru*91 AFLP markers therefore occur more or less on either side of the centromere at 49.2 pu, with the second peak


Genetic Distance cM
Physical distance pu
Genotype
0
0
E32/M40(3)[H],H37/M36(2),H36/M36(2)[L],
H43/M40(5)[L],H43/M40(3)[L],H37/M36(3),RZ783,C112.
9.3
7.1
18
H44/M39(5)[L],
E33/M41(1),
H42/M35(1),E32/M40(4)[H].
16.1
11.4
11
25.7
12.4
H36/M36(3),H35/M39(2),E32/M41(2),H38/M36(2),E38/M40(3)[M],32/M37(2),E36/M37(3)[H].
3/26
H35/M42(1),E33/M37(3)[H].
27.5
15.5
6
H38/M36(1),E34/M35(1),H35/M39(3),
33.4
17.9
3
H42/M35(2)[L],H44/M39(1)[L],E34/M33(2)[M],C949.
34.4
19.7
3/10
H36/M37(3),H37/M33(2)[L],H35/M42(3),H32/M37(1),CDO345.
E32/M41(1), E36/M43(3),H37/M36(1).
36.9
28.9
36
H41/M33(2),H43/M40(2),H36/M37(2),H39/M37(2),
E38/M40(2),H31M40(1),E38/M40(4),H37/33(1),
H43/M40(4)[L],E33/M38(1)[H],E36/M37(1)[H],H36/M36(1)
H43/M40(6)[L],C250,C122,WG889,BCD128.
56.7
38.0
17
H35/M41(2),E33/M37(7),E33/M37(6)[L],
H36/M33(2)[H].
58.9
44.5
83
59.2
47.3
92
H35/M39(4),CDO455.
Centromere
49.2
E39/M38(1),E40/M40(1)[H],H33/M33(1),H35/M39(5)[L],
H38/M43(1),E36/M43(1),H44/M39(4)[L],H44/M39(3),
H41/M33(1)[L],H33/M38(2),H39/M37(1)[H],R1928,
PSR370,RZ382,PSR394,CDO920.
NOR
56.6
60.5
62.5
3/2
E38/M40(1)[M],E32/M41(3)[H],H43/M40(1),E40/M40(2)[L],
E36/46(4),E36/M46(1)[M],E36/M43(4),E36/M37(5)[L],
E36/M37(2)[M],E34/M33(1)[H],E33/M41(2)[H].
61.6
68.5
99
NOR
70.0
H42/M35(3),E34/M32(2)[H],E33/M37(5)[L].
61.9
73.1
83
E39/M38(2)[M],E40/M40(3)[H],H35/M41(1),E38/M32(2)[M],
E39/M32(1)[H],E39/M32(2)[M],H35/M39(1).
63.9
80.0
19
66.9
82.0
56
68.2
82.4
E38/M32(1),E36/M46(3)[M],E39/M32(3)[L],E33/M38(2)[M],R1613.
E33/M37(4)[L].
3/23
H44/M39(2),E36/M43(2),E36/M46(2)[M],E33/M37(2)[M],H35/M42(2),
71.8
86.6
2/3
E36/M37(6)[L],H31/M40(2),E34/M32(3)[H],E33/M41(3),CDO328.
H33/M33(2),H37/M33(3)[L],H36/M37(1)[L],E39/M32(4),
E39/M38(3),H33/M38(1)[H],H42/M35(4),H36/M33(1)[H],
CDO460,C30.
80.9
100

being physically closer to the centromere than the first. Between the two peaks, however, the number of *Hin*dIII/*Tru*91 AFLP markers falls sharply, while the *Eco*RI/*Tru*91 markers fall to zero. A lower number of *Hin*dIII/*Tru*91 AFLP markers than expected, *i.e.*, 7%, was found from 65 to 80 pu along the chromosome. This region coincided with the position of the NOR (56.6–70 pu) and the highest frequency of *Eco*RI/*Tru*91 AFLP markers. Therefore, the distribution of both types of AFLP markers considered together has a sharp fall in numbers at 45 pu along the chromosome with a peak on either side. The peak in AFLPs in the arm without the NOR was made up almost entirely of the *Hin*dIII/*Tru*91 markers, while the peak in the chromosome arm with the NOR was made up of both *Hin*dIII/*Tru*91 and *Eco*RI/*Tru*91 markers.

Each type of marker also falls sharply in a second region of the chromosome. *Eco*RI/*Tru*91 fell to zero at ~19 pu along the chromosome, while *Hin*dIII/*Tru*91 fell to zero at ~81 pu of the distance along the chromosome. Therefore both types of markers fell to zero ~19 pu in from alternate telomeres.

Southern analysis of 32 *Hin*dIII/*Tru*91 and 36 *Eco*RI/*Tru*91 excised and amplified AFLP bands revealed that 18 bands were derived from highly repetitive sequences, 27 bands from moderately repetitive sequences, and 23 bands from low-copy sequences. The distribution of these three classes of AFLP along the Festuca chromosome appeared to be random (Figure 2). For example, of 15 AFLP markers located in the region of the chromosome that carried both the centromere and the NOR (physical location 47.3–73.1 pu), 6 were derived from highly repetitive sequences, 3 from moderately repetitive sequences, and 6 from low-copy sequences.

Variation in the frequency of recombination along the Festuca chromosome: The genetic position of each recombination site was taken to be the midpoint between the last AFLP marker in the previous segment and the first AFLP marker in the following segment. Through the generation of both the genetic (King *et al.* 2002, accompanying article) and physical maps, it was possible to plot how genetic distance varied with physical distance along the length of the Festuca chromosome (Figure 4) to show the variation in recombination frequency.

There are two major peaks in the frequency of recombination, which occur at approximately the same place in each arm although the peak in the NOR arm is considerably smaller than the peak in the non-NOR arm. The single peak in the NOR arm occurs at 18 pu from the telomere, and that in the other arm at ~12 pu from the other telomere. The arm without the NOR also contains two minor peaks, at 18 and 35 pu from the telomere.

Recombination reached its lowest level between 45 and 75 pu along the chromosome. The centromere (positioned at 49.2 pu) and the NOR (positioned between 56.6 and 70 pu) both fall within this region.

RFLP analysis: Eighteen RFLP probes (Figure 2) were placed on the physical map of the Festuca chromosome in line BC_1 57. Of these, 16 were derived from cDNA and 2 from genomic DNA. The 18 RFLP probes were fairly uniformly spread along the whole length of the chromosome, being located in 9 of the 18 segments (Figure 2). These included those segments both containing and adjacent to the centromere and NOR, as well as the more distally placed segments on the physical map (Figure 2). All of these RFLP markers have previously been mapped to rice chromosome 1 (Van Deynze *et al.* 1998). RFLP probes previously mapped to other rice chromosomes did not map to the Festuca chromosome. The data so far obtained appear to indicate that complete macrosynteny has been maintained between the Festuca chromosome and rice chromosome 1.

DISCUSSION

Distribution of recombination sites: The junctions between each of the *F. pratensis* and *L. perenne* segments in the recombinant chromosomes represent sites of recombination. This is in contrast to most other forms of physical mapping that rely on chromosome breakage, *e.g.*, deletion mapping in wheat (Werner *et al.* 1992; Gill *et al.* 1993; Kota *et al.* 1993; Delaney *et al.* 1995a,b; Mickelson-Young *et al.* 1995; Gill *et al.* 1996a,b; Weng *et al.* 2000), translocation mapping in barley (Künzel *et al.* 2000), and physical mapping of maize using the oat-maize radiation hybrids (Riera-Lizarazu *et al.* 1996, 2000; Ananiev *et al.* 1997; Okagaki *et al.* 2001). These types of physical mapping rely on extrapolation between genetic and physical maps to predict the physical position of recombination. The approach used in this article

Figure 2.—Physical map of the Festuca chromosome in the monosomic substitution line BC_1 57. Physical and genetic distances for each segment are shown on the left. Horizontal black arrows indicate sites of recombination between Festuca and Lolium (numbers over the lines show the BC_2 plant from which the segment was obtained). The positions of the centromere (green arrow) and NOR (red arrows) are also shown on the left. Segment allocation of the genetic markers is shown to the right. *Eco*RI/*Tru*91 AFLP markers are shown in black, *Hin*dIII/*Tru*91 AFLP markers in red, and 18 RFLP markers in blue. Alternating blue and yellow colors are used only to aid discrimination between different recombinant lines carrying different-sized segments. For example, BC_2 18 carries a segment 9.3 cM in length and 7.1 pu physical distance with 6 AFLP markers and 2 RFLP markers. Genotype BC_2 11 carries a segment 16.1 cM in genetic length representing 11.4 pu of the physical length and contains an additional 4 AFLP markers to those in genotype BC_2 18. Square brackets at the end of an AFLP indicate that the amplified product is either highly [H] or moderately [M] repetitive or a low-copy sequence [L].

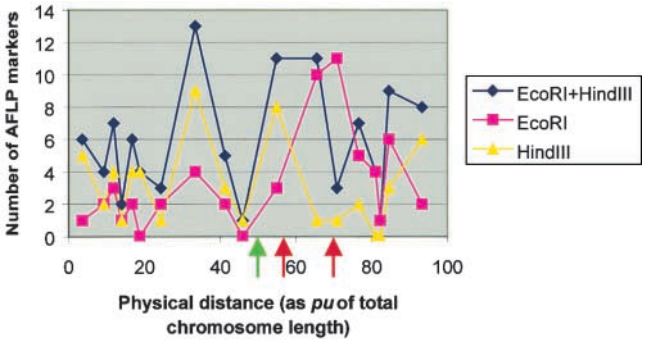


FIGURE 3.—Physical distribution of AFLP markers along the Festuca chromosome present in BC_1 57. The number of AFLP markers present in each Festuca segment has been plotted at the midpoint of each segment. Zero percent physical distance is taken as the telomere of the arm without the NOR. The green arrow marks the position of the centromere while the red arrows mark the position of the NOR.

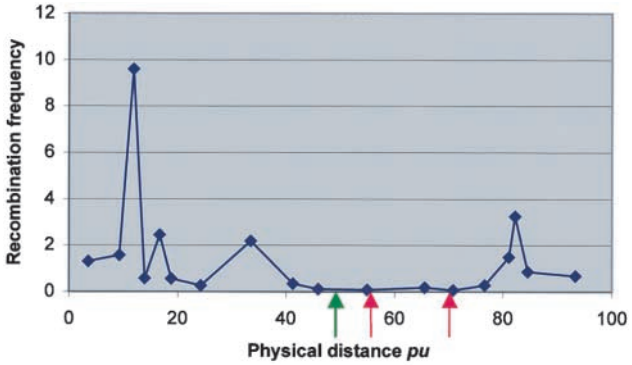


FIGURE 4.—Physical distribution of recombination frequencies along the Festuca chromosome present in BC_1 57. The genetic position in centimorgans of each recombination site was taken to be the midpoint between the last AFLP marker in the previous segment and the first AFLP marker in the following segment. Genetic distance was plotted at the midpoint of each physical segment. The green arrow marks the position of the centromere while the red arrows mark the position of the NOR.

is therefore unique in that crossover positions can be identified in the same individuals both by GISH and mapped genetic markers.

The distribution of recombination sites along the whole length of the chromosome included those very close to the centromere and within the NOR although not between the two. Thus, although the centromere and NOR both cause a reduction in the frequency of recombination in the region between them (see below), recombination itself does take place within these regions.

Two gaps of >10 pu were observed on the physical map of the Festuca chromosome. The distribution of recombination sites along the whole length of the chromosome, however, shows that the present physical map has the potential to be broken down into much smaller sections. To achieve this, BC_2 plants would simply be screened for two AFLP markers, one at either end of the segment that was to be reduced in size. Any BC_2 plants carrying just one of the AFLP markers would therefore have a recombination site within the required region. In regions of lower recombination frequencies, this should prove a very efficient strategy for increasing the saturation of the physical map.

Physical distance compared to genetic distance: A comparison of the physical and genetic maps clearly shows how their interrelationship varies from one part of the chromosome to another. Two gaps on the physical map do not coincide with gaps on the genetic map. In fact, the density of AFLP markers on the genetic map is such that the largest distance between markers is only 5.9 cM and only two other gaps of between 4 and 5 cM are present (KING *et al.* 2002, accompanying article). Of the two gaps on the physical map, the first (a gap of 15.2 pu) contains 11 AFLP markers spread over just 1.3 cM, while the second (a gap of 13.4 pu) contains 8 AFLP markers spread over 9.1 cM.

One of the most obvious differences between the two maps is the relative length of the two chromosome arms. On the physical map the centromere was found to be located virtually in the center of the chromosome. Thus the two arms of the *F. pratensis* chromosome are physically the same size. However, genetically, the non-NOR arm was three times larger (60 cM) than the NOR arm (20.9 cM). The difference in the genetic lengths of the two arms therefore indicates that recombination is more frequent in the arm without the NOR. In addition, GISH applied to meiosis I in pollen mother cells of BC_1 57 allowed the *F. pratensis/L. perenne* bivalent to be visualized and this showed the non-NOR arm to have the greater number of chiasmata (KING *et al.* 2002, accompanying article).

Recombination levels were found to vary within as well as between arms. The lowest frequency was found between 45 and 75 pu of the distance along the chromosome (the region of the chromosome containing both the centromere and the NOR). A reduction in recombination frequency in centromeric regions has been assumed in much of the previous work on genetic mapping, to explain the centromeric clustering of markers. For example, TANKSLEY *et al.* (1992) reported a 10-fold reduction in recombination in the centromeric regions of tomato and potato. This reduction in recombination around the centromere has also been demonstrated in many of the reports where both genetic and physical mapping have been possible, for example, in wheat (CURTIS and LUKASZEWSKI 1991; WERNER *et al.* 1992; GILL *et al.* 1993, 1996a,b; KOTA *et al.* 1993; HOHMANN *et al.* 1994; CHEN and GUSTAFSON 1995; DELANEY *et al.* 1995a,b; MICKELSON-YOUNG *et al.* 1995; WENG *et al.* 2000), rye (LUKASZEWSKI 1992; ALONSO-BLANCO *et al.* 1993), and barley (KÜNZEL *et al.* 2000).

Our results show that the centromere was physically

mapped at 49.2 pu along the chromosome. The frequency of recombination started to increase at a distance of only ~5 pu from the centromere in the arm without the NOR. In contrast, it remained extremely low in the NOR arm for the whole of the region between the centromere and the NOR and including the NOR itself, but rose sharply after the end of the NOR. However, the peak in the NOR arm was considerably smaller than the major peak in the non-NOR arm. This result strongly suggests that the NOR, as well as the centromere, causes a reduction in the frequency of recombination. Similar evidence for little or no crossing over between the centromere and NOR has been reported for chromosomes 1B and 6B of wheat (Dvořák and Chen 1984; Payne *et al.* 1984; Snape *et al.* 1985), barley chromosomes 6 and 7 (Linde-Laursen 1979), and rye chromosome 1R (Lawrence and Appels 1986).

There is a general agreement from work published previously on the grasses that the frequency of recombination tends to be higher in the more distal regions of chromosome arms. Results obtained through deletion mapping in wheat (Werner *et al.* 1992; Gill *et al.* 1993; Kota *et al.* 1993; Hohmann *et al.* 1994; Delaney *et al.* 1995a,b; Mickelson-Young *et al.* 1995) initially suggested a raised frequency in the distal 50 pu of each chromosome arm, with the highest frequency in the most distal 10 pu. Later studies on the deletion lines (Gill *et al.* 1996a,b; Weng *et al.* 2000) continued to report higher frequencies in distal regions relative to the more proximal regions. However, they also identified localized hot spots of recombination in small interstitial regions within the distal 50 pu of the long arm of each chromosome and within the distal 75 pu of each short arm. Using GISH to physically map single-copy RFLP probes, Chen and Gustafson (1995) reported the highest frequency of recombination to be located in the segment 40–65 pu along the chromosome arm from the centromere. In barley, Künzel *et al.* (2000) reported the localization of most of the recombination to a few distinct chromosomal regions. These regions were mostly confined to the ends of the chromosomes but were also found in interstitial positions specific to each of the individual chromosome arms.

The results reported in our work are therefore only partly in agreement with previously published work. The highest frequencies of recombination were found to be located in the distal regions of the chromosome arms, *i.e.*, the two peaks at 18 and 88 pu, respectively. These distances are not as distally located as was initially reported for the deletion mapping in wheat; *i.e.*, they are not within the most distal 10 pu of the chromosome arms. They are in agreement, however, with some of the localized hot spots of recombination reported for wheat homeologous group 5 (Gill *et al.* 1996a) and group 1 (Gill *et al.* 1996b) chromosomes. Five localized hot spots were reported for homeologous group 1, two in the short arms and three in the long arms. One hot spot in each of the arms was located between 10 and 20 pu from the telomeres. These two hot spots were therefore located in the same positions as the two peaks recorded here. The deletion mapping, however, recorded more than one hot spot for each arm. It is possible that the number of hot spots is linked to the genetic length of the chromosome or chromosome arm. In the deletion mapping three hot spots were found in the long arm of group 1 chromosomes but only two in the short arms. In the Festuca chromosome, the non-NOR arm (the longer in terms of genetic distance) did have additional minor peaks in recombination frequency. The second of these minor peaks was located at 35 pu along the chromosome. This position is more proximal than any recorded from deletion mapping in wheat. The NOR arm of the Festuca chromosome had only the one peak, due probably to the reduction in the frequency of recombination in the region of the chromosome surrounding the NOR as well as the centromere. *In situ* hybridization of single-copy RFLP probes (Chen and Gustafson 1995) revealed that the region of wheat homeologous group 7 chromosomes with the highest frequency of recombination is in a more interstitial position than any of the peaks reported here for the Festuca chromosome. The localization of recombination in barley to a few chromosomal regions (Künzel *et al.* 2000) corresponds well to the later deletion mapping work. Again, therefore, there is some agreement between the results obtained in barley and the results reported here. Most of the segments with higher recombination in barley were found in the distal regions of the chromosome arms corresponding to the two highest peaks in the Festuca chromosome. A few of the regions were located in more interstitial regions, but again they were not as proximal as the minor peak recorded in the non-NOR arm of the Festuca chromosome.

Physical distribution of AFLPs: AFLP markers have been used extensively over the last few years in many areas of genetic mapping. They provide good genome coverage, but clustering in centromeric regions is common. Information on the physical distribution of AFLPs within genomes is sparse, with only a few publications on the subject (Meksem *et al.* 1995; Cho *et al.* 1996; Reamon-Büttner *et al.* 1999; Huang *et al.* 2000). King *et al.* (2002, accompanying article) have clearly shown different distribution patterns for *Eco*RI/*Tru*91 AFLP and *Hin*dIII/*Tru*91 AFLPs on the genetic map of the Festuca chromosome. GISH results obtained here for the BC_2 mapping population show that the two types of AFLP markers also have different physical distributions, especially in the location of their clusters.

Ultimately the position of AFLP markers is dependent on the location of the restriction sites of the enzymes used in their production. It must also be remembered, however, that the AFLP markers used here are those for which the band is present only on the Festuca chromosome; *i.e.*, they represent sites of polymorphism be-

tween *F. pratensis* and *L. perenne*. As *Tru*91 was the four-cutter enzyme used in the production of both types of AFLP, different distributions of the two types could therefore suggest a different distribution of *Eco*RI and *Hin*dIII restriction sites or different patterns of inhibition of the two enzymes. *Eco*RI and *Hin*dIII have the same six nucleotides in their restriction sequences (*Eco*RI, 5′-GAATTC-3′; *Hin*dIII, 5′-AAGCTT-3′). The two enzymes also appear initially to be similar in their degree of sensitivity to methylation. Cleavage by *Eco*RI is inhibited by methylation to either of the A nucleotides and also by methylation to the C nucleotide while cleavage by *Hin*dIII is inhibited by methylation of the first A nucleotide and also by methylation to the C nucleotide (McClelland *et al.* 1994). However, while A methylation has not been widely reported in plants, C methylation appears to be abundant and is most consistently associated with the symmetrical sequences CG and CXG (McClelland *et al.* 1994). Thus, of the two enzymes used, only *Eco*RI can have a C as part of a CG dinucleotide or a CXG trinucleotide sequence. As methylation of the C on either strand will block the action of the enzyme, inhibition can occur at a high frequency. It could therefore account for the lack of *Eco*RI markers in regions of the chromosome where *Hin*dIII marker numbers are high, if the *Eco*RI sites are methylation inhibited. However, methylation differences cannot account for the reverse situation where regions of the chromosome have high numbers of *Eco*RI markers but the *Hin*dIII markers are present in extremely low numbers.

The clusters of Festuca-specific AFLP markers on the physical map suggest that the enzymes used to produce the markers are restricting the DNA at numerous sites within the same region of DNA; *i.e.*, the enzymes may be cutting frequently in a region of repetitive DNA carrying Festuca-specific repeats. *Eco*RI/*Tru*91 AFLP markers were clustered throughout the region of the chromosome containing the NOR (presumably in the less conserved spacer regions). In contrast the *Hin*dIII/*Tru*91 AFLP markers are clustered around the centromere (also known to carry large amounts of repetitive DNA). If this were the case it might be expected that AFLP bands from the clusters would consist mostly of repetitive sequences. However, Southern hybridization of genomic Festuca DNA to blots of the excised and amplified AFLP bands does not agree with this hypothesis; *i.e.*, AFLP bands from clustered regions were derived from a mixture of low, moderate, and highly repetitive sequences (Figure 2).

Physical distribution of RFLP markers: Deletion mapping in wheat has suggested that most genes are found in clusters. These gene-rich regions are recombination hot spots, which make up very small physical distances (~10%) and are separated by large marker-poor regions. Until the recently published work on wheat group 1S chromosomes (Sandhu *et al.* 2001), no markers were found in the region surrounding the centromeres (Gill *et al.* 1996a,b). Translocation mapping in barley (Künzel *et al.* 2000) produced a very similar series of results to those obtained by the deletion mapping in wheat. The majority of the RFLP probes (both cDNA and genomic) were located in a few, relatively small areas per chromosome also characterized by high frequencies of recombination. These small physical distances were interspaced by much larger, marker poor regions with extremely low frequencies of recombination. Again no markers were mapped to the centromeric regions.

The physical mapping of the cDNA probes and the linked position of agronomically important genes through deletion mapping led to two possibilities:

1. Genes, especially in the larger genomes, are found in clusters, which also appear to have high frequencies of recombination. The regions between the gene clusters represent spacer regions composed mostly of repetitive DNA.
2. Highly conserved genes (housekeeping genes) may be present in recombination poor regions of chromosomes while less conserved genes may be present in regions of higher recombination frequencies [a theory originally put forward to explain the distribution of genes in the human genome (Mouchiroud *et al.* 1991)]. Deletion mapping has tended to eliminate the second possibility.

Therefore the data in this study tend to contradict some of the earlier results from the physical mapping of wheat and barley. As with the results obtained on the group 1S chromosomes of wheat (Sandhu *et al.* 2001), the physical map of the Festuca chromosome clearly locates cDNA RFLP probes in very close proximity to the centromere. This obviously suggests the presence of genes in this region. Differences in gene densities have been found to vary on the chromosomes of Arabidopsis but expressed gene sequences have been shown to be located within the centromere (Copenhaver *et al.* 1999).

The RFLP data are also interesting from the point of view of the synteny shown between the whole length of the Festuca chromosome and rice chromosome 1 and hence through comparative genome analysis to the chromosomes of other grass species, *e.g.*, wheat homeologous group 3 (Van Deynze *et al.* 1998). Work on synteny within the grasses has previously shown blocks of synteny between species interrupted by, for example, inversions or translocations (Moore 1995; Moore *et al.* 1995a,b). Importantly the blocks require rearranging to form the other genomes. This is not the case with Festuca and rice. Although it is possible that minor differences might come to light with a higher density of RFLP probes, the Festuca chromosome presently appears to be completely homeologous to rice chromosome 1.

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