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# Genome mapping of polyploid tall fescue (*Festuca arundinacea* Schreb.) with RFLP markers

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**Abstract** Genetic mapping using molecular markers such as restriction fragment length polymorphisms (RFLPs) has become a powerful tool for plant geneticists and breeders. Like many economically important polyploid plant species, detailed genetic studies of hexaploid tall fescue (*Festuca arundinacea* Schreb.) are complicated, and no genetic map has been established. We report here the first tall fescue genetic map. This map was generated from an $F_2$ population of HD28-56 by 'Kentucky-31' and contains 108 RFLP markers. Although the two parental plants were heterozygous, the perennial and tillering growth habit, high degree of RFLP, and disomic inheritance of tall fescue enabled us to identify the segregating homologous alleles. The map covers 1274 cM on 19 linkage groups with an average of 5 loci per linkage group (LG) and 17.9 cM between loci. Mapping the homoeologous loci detected by the same probe allowed us to identify five homoeologous groups within which the gene orders were found to be generally conserved among homoeologous chromosomes. An exception was homoeologous group 5, in which only 2 of the 3 homoeologous chromosomes were identified. Using 12 genome-specific probes, we were able to assign several linkage groups to one of the three genomes ($PG_1G_2$) in tall fescue. All the loci detected by the 11 probes specific to the $G_1$ and/or $G_2$ genomes, with one exception, identified loci located on 4 chromosomes of two homoeologous groups (LG2a, LG2c, LG3a, and LG3c). A P-genome-specific probe was used to map a locus on LG5c. Comparative genome mapping with maize probes indicated that homoeologous group 3 and 2 chromosomes in tall fescue corresponded to maize chromosome 1. Difficulties and advantages of applying RFLP technology in polyploids with high levels of heterozygosity are discussed.

**Key words** RFLP · Genetic map · Polyploids · Tall fescue · Molecular marker

## Introduction

Genetic mapping using molecular markers such as restriction fragment length polymorphisms (RFLPs) has become a powerful tool for plant geneticists and breeders. Genetic linkage maps with molecular markers have been informative and useful in assaying genetic variation within a plant species (O'Donoughue et al. 1994; Xu et al. 1994). Disease resistance genes can be localized by linkage analysis, cloned on the basis of the linkage map, and transformed to produce transgenic resistant plants (Martin et al. 1993). Quantitative trait loci (QTLs) can be dissected, tagged, and used in marker-assisted selection to improve agronomically important traits (Dudley 1993; Paterson et al. 1988; Beavis et al. 1991; Anderson et al. 1993). Recently, comparative genome mapping has provided new insights into the organization and evolution of plant genomes (Bennetzen and Freeling 1993; Ahn et al. 1994).

Many economically important plant species are polyploids, as are about 30–70% of the angiosperms (Stebbins 1971; Masterson 1994). It is perhaps in these polyploid species that DNA genetic markers and linkage maps are needed, even more than for species for which genetic information is abundant, to provide information for scientific study and genetic manipulation. However, the genetic mapping of polyploids has lagged behind diploids due to such factors as the presence of a large number of segregating genotypes, poorly characterized genome constitutions and chromosome pairing, and complicated genotype characterization due to the

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segregation of multiple fragments and co-migration of different fragments (Sorrells 1992).

At the present time no genetic map (molecular or otherwise) is available for tall fescue. Tall fescue is widely used as a cool-season perennial forage and turf grass. It contains three genomes ($PG_1G_2$), one from *F. pratensis* (2x, P genome) and two from *F. arundinacea* var *'glaucescens'* (4x, $G_1G_2$ genomes) (Sleper 1985), with a genome size of approximately $5.27–5.83 \times 10^6$ kb (Seal 1982). The allohexaploid (2n = 6x = 42) and outcrossing nature of tall fescue causes difficulties in genetic analyses and provides a stimulating challenge in the breeding of improved cultivars because allelic variation, genome constitution, and ploidy level all influence phenotypic expression (Sleper 1985).

We previously developed a *Pst*I genomic DNA library with DNA from a hexaploid tall fescue plant and found that tall fescue is highly polymorphic. The average degree of polymorphism detected by *Pst*I clones was 70%, and genome-specific probes were also identified (Xu et al. 1991). We have used these *Pst*I genomic clones to construct an RFLP-based map. To facilitate future comparative mapping studies, we have included a few maize RFLP markers in the mapping analysis. We report here the first RFLP linkage map of tall fescue and a strategy to deal with the polyploid nature and heterozygosity of parental lines.

## Materials and methods

### Plant materials

An $F_2$ population was generated from a cross between two highly polymorphic hexaploid tall fescue plants. The female parent was HD28-56, a plant characterized as having high forage quality; the male plant was from the cultivar 'Kentucky-31', the most widely grown cultivar in the United States. Because the parents came from different genetic backgrounds, considerable allelic differences were expected. The two plants were crossed through emasculation and hand pollination to produce $F_1$ plants. Seven $F_1$ plants were confirmed to be hybrids by RFLP analysis, grown in the field, and transplanted into the greenhouse after vernalization. During anthesis, each $F_1$ plant was placed in isolation in a different greenhouse room and then selfed to produce $F_2$ seeds. As the parents were not inbred lines, each $F_1$ plant produced a different $F_2$ population. One of the $F_2$ populations consisted of 180 seeds and was chosen for mapping. Seeds were germinated in petri dishes, and individual $F_2$ plants were grown in the greenhouse. The seeds germinated at different rates, therefore 105 of the $F_2$ plants were randomly chosen for mapping to eliminate the effect of seedling vigor. Leaf samples were harvested from plants in the greenhouse for RFLP analysis.

### RFLP analysis

The tall fescue genomic clones were from a *Pst*I genomic library developed in our laboratory and designated with a TF prefix and laboratory number. Additionally, three maize probes that identify loci located on maize chromosome 1 (one genomic: UMC167, and two cDNAs: CSU3 and CSU92) were also included. Probes were screened using three enzymes (*Bam*HI, *Eco*RI, and *Hin*dIII). The most informative probe-enzyme combination was chosen for mapping analysis. The mapping blots contained genomic DNAs from the female parent (HD28-56), male parent ('Kentucky-31'), $F_1$ and 105 $F_2$ plants. The procedures used for RFLP analysis have been described previously (Xu et al. 1991).

### Nomenclature and map construction

A locus detected by a particular RFLP probe was designated after the probe except that prefix letters were presented in lower-case print. Due to the polyploid nature of tall fescue, the majority of the probes detected multiple segregating fragments. In this case ascending letters (i.e., A, B, C, D, etc.) were given to each of the segregating fragments according to their sizes and added to the probe name as a suffix. Each fragment was first scored as a dominant allele, and the allelism with other fragments was later determined (see Results and Discussion). The homologous allelic fragments were then combined and scored as a single co-dominant locus for linkage analysis.

A linkage map was constructed using multi-point maximum likelihood methods as performed by the computer program MAPMAKER (Macintosh v. 2.0) (Lander et al. 1987). The criteria for odds of marker order were at least 1000:1, and the maximum recombination ratio for linkage was 40%. The recombination frequencies were converted to centiMorgans (cM) by the Haldane mapping function (Haldane and Waddington 1931).

## Results

### Strategy of determination of homoeoalleles in tall fescue

Genetic analyses in tall fescue is supposedly complicated by its polyploid nature and the presence of high levels of heterozygosity. However, these complicating features may be beneficial when coupled with RFLP analyses. A high level of polymorphism (over 70%) was detected between different tall fescue lines using randomly cloned DNA fragments as probes. In most cases, polymorphic probes detected multiple segregating fragments, thus 2–3 loci could usually be mapped using a single probe (Fig. 1). For example, probe TF49 detected four major segregating fragments in which allelic fragments were to be identified by further analysis. In addition, segregating fragment B was present in both parents, and its parental origin was not apparent. Moreover, because an $F_2$ population was used to generate the RFLP map, the mapping of dominant markers will be inefficient, especially for closely linked loci. Therefore, to make the mapping more efficient with this and other probes, we followed several procedures. First, we scored each segregating fragment as a dominant marker (i.e., presence or absence). Fragment B was scored as a dominant marker of either female origin (like fragment A) or male origin (like fragment C). The segregation data obtained with probe TF49 was analyzed separately using MAPMAKER's Two Point/Group command, followed by applying the Three Point/Map command. When this approach was used fragments located on different homoeologous chromosomes were expected to be independent, while allelic fragments were expected to show co-segregation. As a result, fragments A and C were placed in one group and mapped at the same location, indicating they were allelic to each other, and fragment B (contrib-

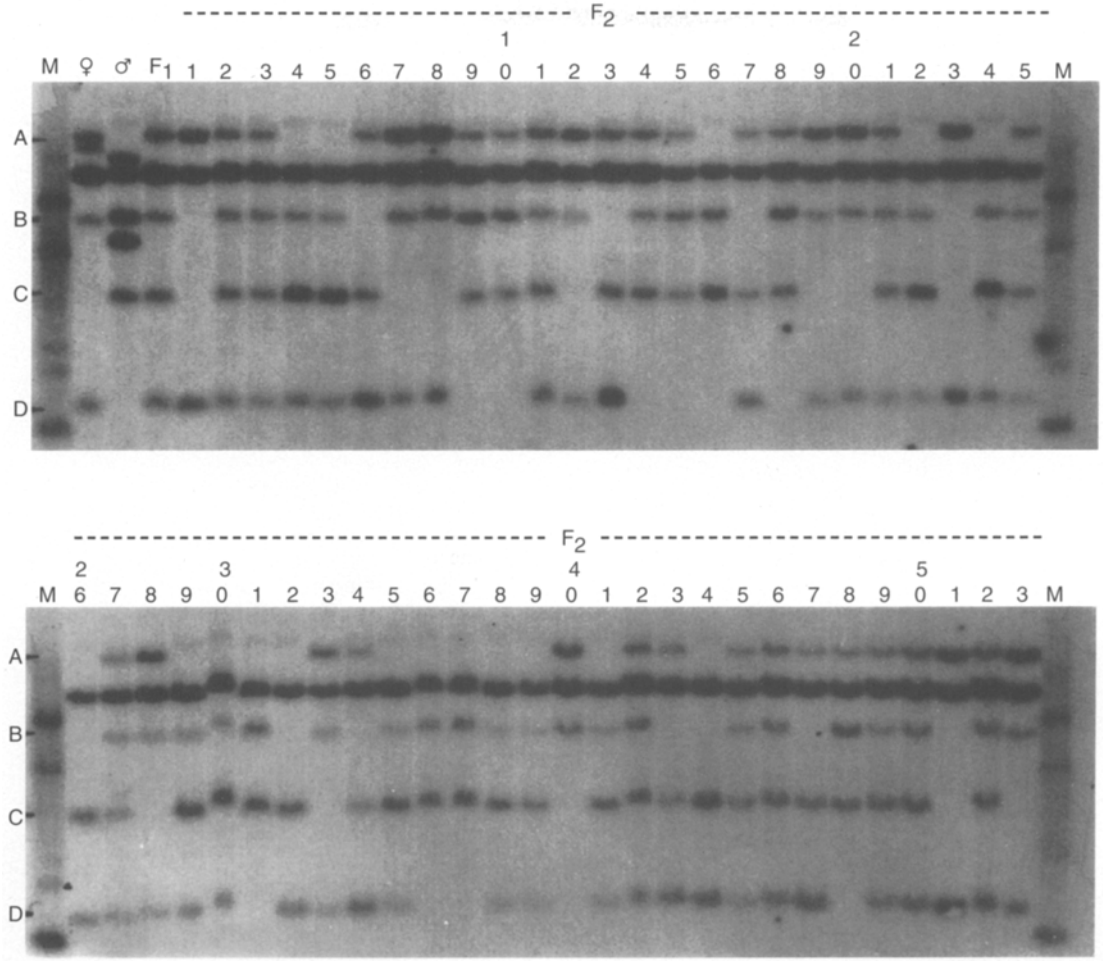



**Fig. 1** $F_2$ segregation detected with probe TF49. Two loci are segregating, ft49AC (i.e. fragments *A* and *C* are allelic) and tf49BD. DNA was digested with *Hin*dIII. ♀ HD28-56, ♂ 'Kentucky-31'. Only results from 53 of the 105 $F_2$ plants are shown here

uted from the male parent) was allelic to fragment D. When the dominant B fragment was treated as originating from the female, it was placed in a separate group by itself. The locus defined by the A and C fragments was designated as tf49AC, and the locus defined by B (from the male parent) and D fragments as tf49BD. These 2 loci were mapped on different linkage groups, indicating that they were homoeologous to each other. In this manner, we were able to score most of the segregating fragments as co-dominant markers. Those that failed to show allelism with other fragments were treated as dominant markers.

The above analysis of homologous alleles was based on the fact that tall fescue is an allohexaploid and shows disomic inheritance. Disomic inheritance in tall fescue was indicated by earlier cytogenetic (Sleper 1985) and isozyme studies (Lewis et al. 1980), and our RFLP mapping data have also provided additional clear and strong evidence for this mode of inheritance. Segregation at 1, 2, and 3 loci have been observed (Fig. 1). However, the three genomes of tall fescue are not identical (Sleper 1985). When we used the same mapping approach as described above, a probe, TF102, detected homologous sequences on only one chromosome, indicating that this probe is specific to one genome (Fig. 2).

## Segregation of RFLPs and allopolyploidy of tall fescue

As discussed previously, tall fescue is an allopolyploid species. In order to determine if RFLP markers assayed in this study would segregate in expected Mendelian ratios, i.e., 3:1 (presence or absence) for the dominant marker and 1:2:1 for co-dominant markers, Chi-square tests were performed. The results indicated that 73% of the markers followed a normal Mendelian segregation ratio. For example, the 2 loci, tf49AC and tf49BD, detected by probe TF49 as shown in Fig. 1 segregated in a 1:2:1 ratio. The observed ratio for locus tf49AC was 27♀:50 H (heterozygous):28 ♂, and 22♀:56 H:27♂ for the locus tf49BD compared to the expected ratio of 26♀:53 H:26♂.

Segregation for 27% of the markers was skewed, with the dominant loci being the ones most likely to be skewed (Table 1). Twelve out of 27 such loci were skewed

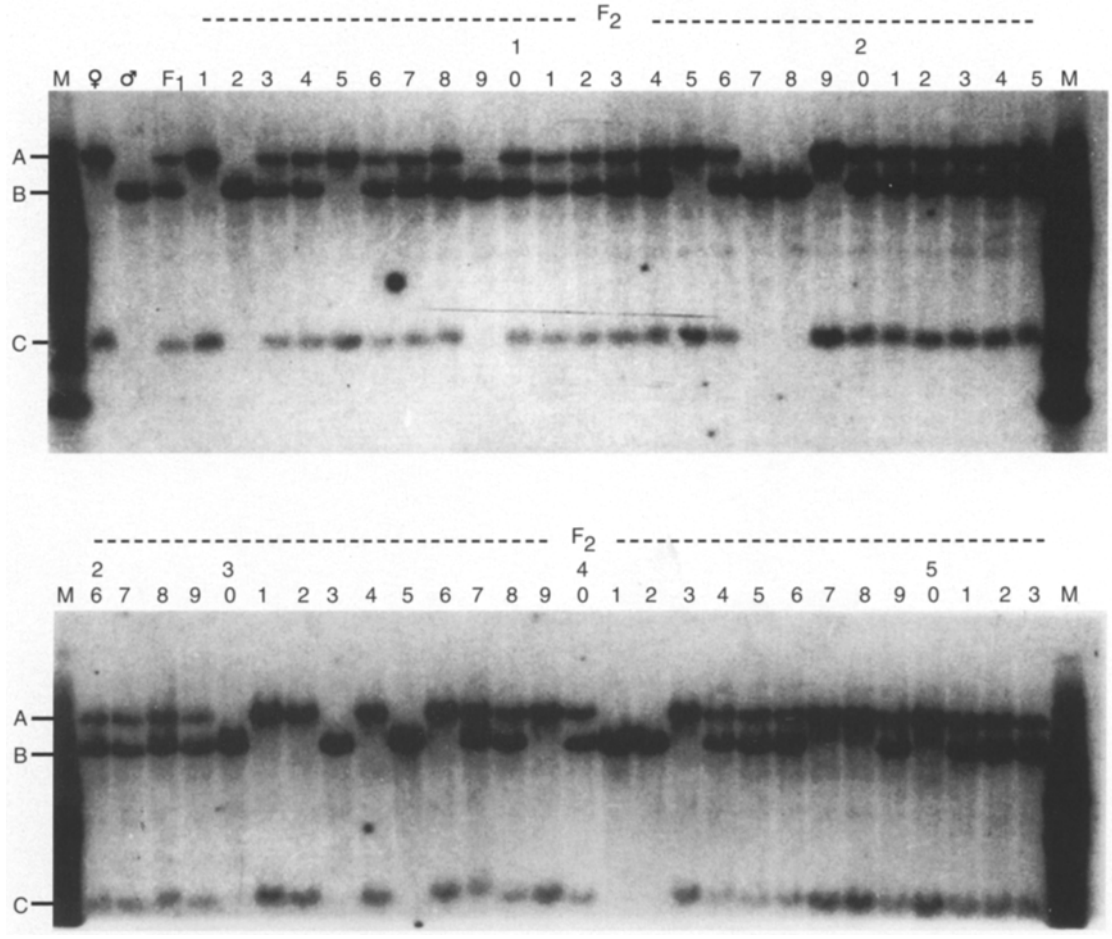



**Fig. 2** $F_2$ segregation detected with probe TF102. Alleles at 1 locus are segregating. Fragments *A* and *C* are from an internal cut and belong to the same allele. Genomic DNA was digested with *Eco*RI. ♀ HD28-56, ♂ 'Kentucky-31'. Only results from 53 of the 105 $F_2$ plants are shown here

compared to 20% of those with the 1:2:1 expected ratio. Because 5 dominant markers from the female and 7 from the male showed distorted segregation, skewness was not determined to favor alleles from a particular parent. At co-dominant loci, heterozygous genotypes were generally favored (Table 1).

## RFLP linkage maps and identification of homoeologous groups

Results from linkage analysis of 108 markers allowed us to construct the first RFLP map for tall fescue, which contained a total of 95 markers. Of the possible 21 linkage groups we found 19, covering a map distance of 1274 cM (Fig. 3); 13 markers could not be identified with any of the linkage groups mapped so far.

Many probes revealed segregation at more than 1 locus (Fig. 1), which allowed us to identify linkage groups that belonged to the same homoeologous group (Fig. 3). Among the 19 linkage groups, we were able to identify at least five homoeologous groups out of the theoretical seven. Homoeologous group 5 has 1 chromosome unidentified in this study. These homoeologous groups were named as 1a, 1b, 1c,..., 4a, 4b, 4c, and 5a and 5b. Letters were used to designate homoeologous linkage groups and were arbitrarily assigned and not associated with a specific genome. Homoeologous chromosomes were found to share high levels of gene synteny (Fig. 3).

The longest linkage group (LG) was LG2a with 12 loci spanning 267.4 cM. On average, each linkage group had 5 loci with a separation of 17.9 cM between adjacent loci (Table 2). Five of the linkage groups had only 2 loci mapped. Linkage groups 15–19 were not placed in any of the homoeologous groups, although LG15 contained tf543AB, csu3CD, and tf45 and their homoeoloci have been mapped on LG2b and LG2c. Therefore, LG15 was recognized as a portion of LG2a, and more markers are needed to link these two segments. By the same token, LG16, LG18, and LG19 all have homoeoloci mapped on LG2a, thus more markers are needed to place these 3 linkage groups together with either LG2b or LG2c.

## Use of genome-specific probes for genome assignment

Although gene synteny was found among three genomes (P, $G_1$ and $G_2$), these three genomes were not identical.

**Table 1** RFLP loci with skewed segregation ratios

| Locus | Linkage group[a] | Ratios[b] (♀:H:♂) | Locus | Linkage group[a] | Ratio (♀:H:♂) |
|---|---|---|---|---|---|
| Toward ♀, ♂ | | | Toward ♀ &4/or H | | |
| tf153 | 5a | 43:16:46 | tf168D | 1b | –:83:21 |
| tf179DE | 5a | 39:34:32 | tf173 | 1c | –:94:11 |
| tf149BD | ? | 43:17:44 | tf202E | 1c | –:91:14 |
| tf205BD | ? | 24:39:42 | tf214A | 2c | –:84:21 |
| Toward H | | | tf227E | 3b | –:85:20 |
| tf165BD | 1c | 33:59:13 | tf235C | 3c | –:87:18 |
| tf428CD | 1c | 33:60:10 | tf215B | 4c | –:78:14 |
| tf570AB | 1c | 31:65:9 | Toward ♂ &/or H | | |
| tf199AB | 2a | 20:68:17 | tf165C | 1a | 18:87:– |
| ft226CD | 2a | 23:65:17 | tf168C | 1a | 22:81:– |
| tf33AB | 3b | 28:68:9 | tf127C | 1c | 25:80:– |
| tf144BC | 3b | 12:73:20 | tf222E | 1c | 17:88:– |
| tf30AB | 4a | 3:93:9 | tf42A | 2b | 25:80:– |
| tf243BC | 4a | 21:78:6 | | | |
| tf249BE | 4c | 8:66:31 | | | |
| tf43 | ? | 17:68:20 | | | |
| tf225CF | ? | 27:72:6 | | | |

[a] The unlinked loci are marked with a question mark
[b] H, Heterozygous genotypes; ♀, female-parent (HD28-56) genotype; ♂, male-parent ('Kentucky-31') genotype; –, dominant marker

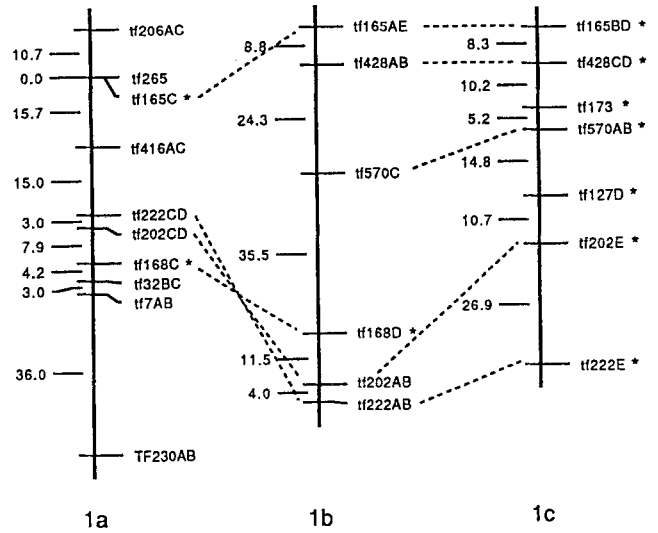
We previously identified clones that were specific to certain genomes by hybridizing genomic clones originating from hexaploid tall fescue (6x, $PG_1G_2$ genomes) to genomic DNAs from tall fescue and its two progenitor species, *F. pratensis* (2x, P genome) and *F. arundinacea* var '*glaucescens*' (4x, $G_1G_2$ genomes) (Xu et al. 1991). In the present study 14 genome-specific probes were also included for RFLP mapping analysis (Fig. 3; Table 3). These genome-specific probes hybridized with one or occasionally to two of the three genomes in tall fescue. The scoring and analysis of the segregation results from these probes were as simple as those conducted in diploids (Fig. 2). Segregation ratios are presented in Table 3. The mapping of these clones should be especially useful for sorting the 21 linkage groups into seven homoeologous groups where three homoeologous chromosomes are from the P, $G_1$, and $G_2$ genomes. On the basis of the map location of these probes, one can determine to which genome a linkage group belongs. For example, TF153, a P-genome-specific probe, was mapped to LG5a; hence LG5a is associated with the P genome. The use of probes specific to the $G_1/G_2$ genomes, i.e., those probes that only cross-hybridized to the genomic DNA from *F. arundinacea* var '*glaucescens*' (4x, $G_1G_2$), allowed us to further distinguish the linkage groups associated with the $G_1/G_2$ genomes as compared to those associated with the P genome. Markers tf27, tf102AB, and tf515A were found to be located on LG2a, and markers tf164BC, tf214A, and tf318 were found on LG2c; therefore, LG2a and LG2c must be associated with either the $G_1$ or $G_2$ genomes. Similarly, LG3a (according to tf248D, tf301, and tf569AC) and LG3c (tf235A and tf544) belonged to the $G_1/G_2$ genomes. None of the $G_1/G_2$-genome-specific markers were mapped on LG2b and LG3b, indicating that LG2b and LG3b were associated with the P genome.

### Mapping of maize RFLP probes and comparative mapping

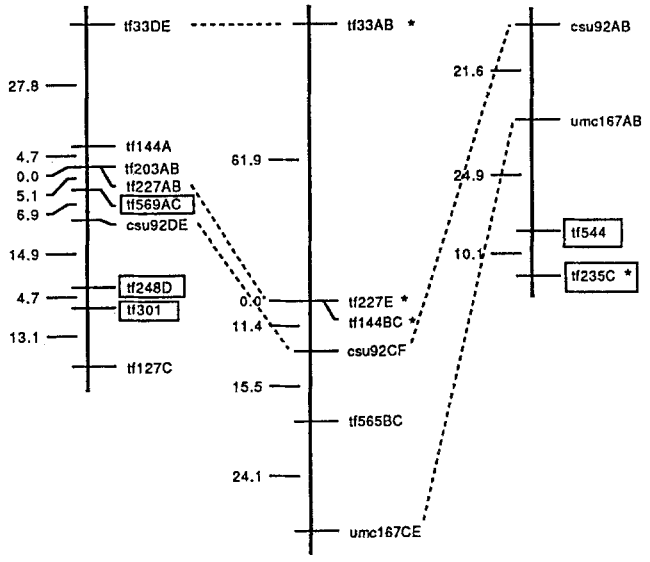
Two maize mature leaf cDNA clones (CSU3 and CSU92) and one maize genomic clone (UMC167) that identified maize chromosome 1 loci (Chao et al. 1994) were used for mapping. In tall fescue, both UMC167 and CSU3 revealed segregation at 2 loci while CSU92 showed segregation at 3 loci. Three homoeologous loci detected by CSU92 and 2 detected by UMC167 were placed on the chromosomes of homoeologous group 3 (LG3a, LG3b, and LG3c) in our tall fescue map, but unlike maize, locus csu3AB detected by CSU3 was located on separate linkage groups LG2b and LG15 (possibly a portion of LG2a).

## Discussion

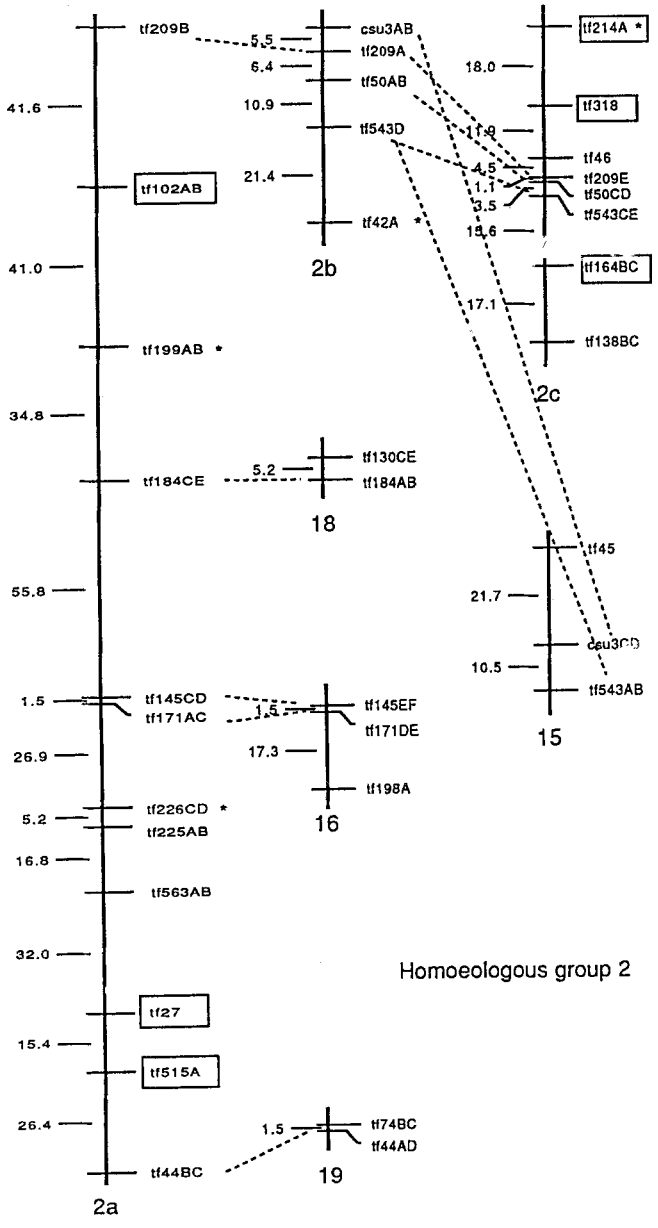
One of the barriers to the genome mapping of tall fescue and many other outcrossing species is the lack of inbred lines. When parents are heterozygous, the polymorphic fragments between them may not always be inherited in the $F_1$ (Fig. 1), and the parental origin of segregating fragments may sometimes be inapparent. Consequently, we included DNAs from the $F_1$ in the screening blots to ascertain mappable polymorphic fragments. The difficulties associated with heterozygosity can be overcome by a co-dominant RFLP marker system and by making use of the perennial growth habit and high levels of RFLPs in these species.



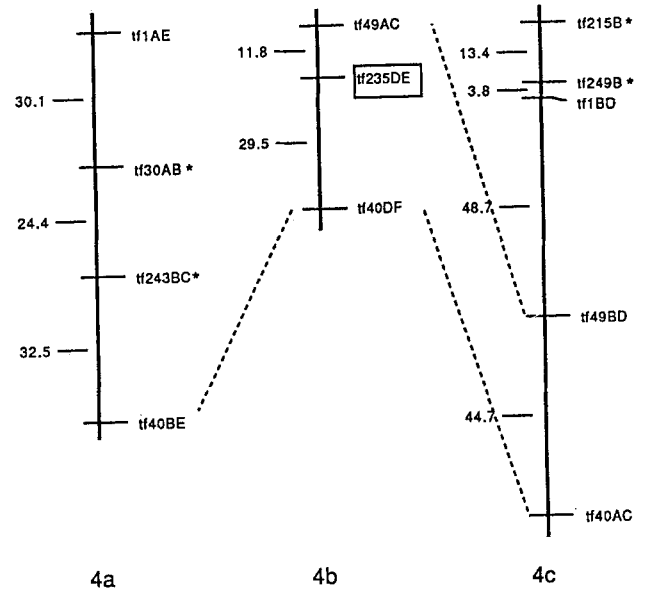

Homoeologous group 1



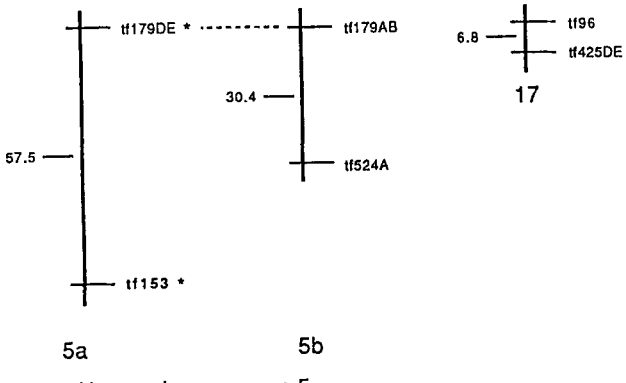

Homoeologous group 3




Homoeologous group 2




Homoeologous group 4




Homoeologous group 5

**Fig. 3** RFLP-based genetic map of tall fescue. The markers specific to $G_1$ and/or $G_2$ genomes are presented in *boxes*, while those specific to the P genome are in *boldface*. Homoeologous loci detected by the same probe are aligned with *dashed lines*. * denotes the markers with distorted segregation ratios

**Table 2** Total number of mapped loci on, and length of, 19 linkage groups

| Linkage group | Number of loci | Total length (cM) | Average distance between loci (cM) |
|---|---|---|---|
| 1a | 10 | 95.5 | 10.6 |
| 1b | 6 | 84.1 | 16.8 |
| 1c | 7 | 72.9 | 12.2 |
| 2a | 12 | 267.4 | 24.3 |
| 2b | 5 | 44.2 | 11.1 |
| 2c | 8 | 71.7 | 10.2 |
| 3a | 9 | 77.2 | 9.7 |
| 3b | 6 | 112.9 | 22.6 |
| 3c | 4 | 56.6 | 18.9 |
| 4a | 4 | 87.0 | 29.0 |
| 4b | 3 | 41.3 | 20.7 |
| 4c | 5 | 110.6 | 27.7 |
| 5a | 2 | 57.6 | 57.6 |
| 5b | 2 | 30.4 | 30.4 |
| 15 | 3 | 32.2 | 16.1 |
| 16 | 3 | 18.8 | 9.4 |
| 17 | 2 | 6.8 | 6.8 |
| 18 | 2 | 5.2 | 5.2 |
| 19 | 2 | 1.5 | 1.5 |
| Total | 95 | 1273.9 | – |
| Mean | 5 | 67.0 | 17.9 |

Another factor that hampers the genome mapping of polyploids is their complicated genotypic characterization due to the segregation of multiple fragments and co-migration of different fragments (Sorrells 1992). Several strategies have been proposed to deal with these problems. Wu et al. (1992) described the use of a single-dose restriction fragment (SDRF) where the segregation of each fragment was analyzed according to its presence or absence in the progeny. However, the mapping of dominant markers in an $F_2$ population is inefficient, especially for closely linked loci. In our study, we ran the gel to the 10-cm line and obtained good resolution. The co-migration of fragments may be solved by running the gel for a longer distance. The strategy outlined in the Results section enabled us to score most of the multiple segregating fragments as co-dominant markers.

While multi-fragment (or loci) segregation posed a challenge for identifying homologous alleles, it can be advantageous because 2 or 3 loci can be mapped simultaneously. The results allowed us to compare maps of homoeologous chromosomes and provided additional information on the evolutionary relationships among the three genomes in tall fescue. The homoeologous loci detected by a probe allowed us to classify 14 chromosomes into five homoeologous groups.

Homoeologous chromosomes were found to share high levels of gene synteny (Fig. 3). However, they are not identical because genome-specific probes have been identified (Xu et al. 1991). Fourteen such probes were mapped in this study. The results shown in Fig. 2 further

**Table 3** Genome-specific probes used for mapping in this study and the segregation ratio of the alleles

| Probe | Genome specificity [a] | | Enzyme [b] | Number of fragments [c] | | | Segregation ratio [d] | | | $\chi^2$ | Linkage group [e] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2x | 4x | | T | S | L | ♀ | ♂ | H | | |
| TF027 | – | + | E | 2 | 2 | 1 | 29 | 28 | 48 | 0.79 | 2a |
| TF102 | – | + | E | 3 | 3 | 1 | 26 | 18 | 61 | 3.97 | 2a |
| TF125 | – | + | B | 2 | 2 | 1 | 28 | x | 77 | 2.10 | ? |
| TF153 | + | – | B | 2 | 2 | 1 | 43 | 46 | 16 | 50.90* | 5a |
| TF164 | – | + | B | 3 | 2 | 1 | 25 | 26 | 48 | 0.11 | 2c |
| Tf214 | – | + | H | 2 | 1 | 1 | x | 21 | 84 | 8.40* | 2c |
| TF235 [f] | – | + | H | 6 | 3 | 2 | x | 18 | 87 | 12.39* | 3c |
| TF235 [g] | – | + | H | 6 | 3 | 2 | 30 | 20 | 55 | 2.14 | 4b |
| TF242 | – | + | B | 3 | 2 | 1 | 30 | 18 | 57 | 3.51 | ? |
| TF301 | – | + | H | 3 | 2 | 1 | 27 | 24 | 54 | 0.26 | 3a |
| TF318 | – | + | E | 2 | 2 | 1 | 23 | 24 | 58 | 1.17 | 2c |
| TF248 | – | + | E | 3 | 1 | 1 | 30 | x | 74 | 0.95 | 3a |
| TF515 | – | + | H | 7 | 4 | 1 | 34 | x | 71 | 0.04 | 2a |
| TF544 | – | + | H | 3 | 2 | 1 | 24 | 23 | 58 | 1.17 | 3c |
| TF569 | – | + | H | 4 | 3 | 1 | 27 | 22 | 56 | 0.94 | 3a |

[a] Genome-specific probes were identified on the basis of their Southern hybridization with DNA from two progenitor species of tall fescue (Xu et al. 1991). 2x = diploid *Festuca pratensis* (genome constitution PP), 4x = tetraploid *Festuca arundinacea* var '*glacescens*' (genome constitution $G_1G_1G_2G_2$). – = The probe did not hybridize to the DNA; + = the probe hybridized to the DNA. TF153 was specific to the P genome; the remainder of the listed probes were specific to the $G_1$ and/or $G_2$ genome (s)

[b] The restriction enzyme used for mapping the probe: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII

[c] T, The total number of fragments detected by the probes in the $F_1$ plant; S, the number of segregating fragments in the $F_2$ population; L, the number of loci to which the segregating fragments were mapped

[d] The segregation ratio of genotypes in the $F_2$ population. A x indicates a dominant marker, and the loci at which significantly a skewed nature occurred are indicated with a * after the $\chi^2$ value

[e] The unlinked loci are indicated by a question mark

[f] Probe TF235 detected 2 segregating loci; f represents locus TF235C

[g] Represents locus tf235DE

corroborated the fact that the three genomes are different from each other and that mapping of the genome-specific probes can help to associate specific linkage groups to specific genomes. The genome-specific probes hybridized only with one or two genomes, and in most cases detected the segregation of 1 locus (Table 3). One exception is TF235, a $G_1/G_2$-genome-specific probe. TF235 detected fragments mapped onto 2 linkage groups, LG3c and LG4b, which belong to two different homoeologous groups. There are two ways of interpreting this result. One possibility is that both LG3c and LG4b belong to the same genome, either $G_1$ or $G_2$, and that TF235 has detected duplicate sequences located on 2 different chromosomes in the same genome. Alternatively, LG3c and LG4b may belong to two different genomes, i.e., LG3c may correspond to a chromosome from the $G_1$ genome and LG4b from the $G_2$ genome, or vice versa. Chromosome deletion or rearrangement during evolution might have taken place to give rise to the present situation in that the homologous sequences located on the two different genomes could be detected by the same probe. If the latter mechanism is true, the neighboring RFLP markers should have also been included in the rearrangement. Our results did not support this; therefore, it is likely that LG3c and LG4b belong to the same genome and that TF235 has detected a duplicated locus.

It is also interesting to note that 11 of the 12 $G_1$- and or $G_2$-genome-specific loci were located on four linkage groups, LG2a, LG2c, LG3a, and LG3c. So far, most of the genome-specific probes identified are derived from the $G_1$ and/or $G_2$ genomes. The mapping of genome-specific RFLP markers not only simplifies segregation analysis in polyploids but also provides a useful tool for genome identification. In addition, chromosome segments marked by genome-specific probes reveals the extent of sequence divergence involved in the P, $G_1$, and $G_2$ genomes during the evolutionary process.

Chromosome rearrangement and/or sequence divergence may have contributed to genome specificity during evolution. Evidence of possible chromosome rearrangement can be found on homoeologous group 1 chromosomes, in which LG1a contains a segment flanked by tf220CD and tf168C that appears to be inverted when compared with LG1b. It is clear that as the resolution of the RFLP map is improved, we should gain a better understanding of how the three genomes are organized in tall fescue.

RFLP loci with distorted segregation have been detected in the mapping populations of many plant species. In this study, 27% of the markers were skewed, in particular, the dominant markers. The distortion percentage varies among species and populations. Distortion has been observed to range from 0% (Beavis and Grant 1991) to 11% (Gardiner et al. 1993) in maize $F_2$ populations; to average 9% in a common $F_2$ bean population (Nodari et al. 1993), 18% in a $F_2$ rice population (McCouch et al. 1988) most of the loci in a $F_7$ recombinant inbred line population (Wang et al. 1994), 50% in a diploid $F_2$ alfalfa population (Brummer et al. 1993), and 14% in a barley doubled haploid population (Kleinhofs et al. 1993).

The distortion may be caused by various processes including gametic and/or chromosome pairing factors, parents exhibiting favored allelic responses after in vitro culture during the development of doubled haploid lines (Gardiner et al. 1993), an association between heterozygosity and plant vigor as found in alfalfa (Brummer et al. 1993), and natural selection toward one parental type during recombinant inbred line development (Wang et al. 1994). In tall fescue and other grasses, self-incompatibility is gametophytic and controlled by genes at 2 loci (Leach and Hayman 1987). Genes linked to the incompatibility loci may give distorted segregation ratios. If gametophytic incompatibility is involved, we could produce $F_2$ seeds by selfing $F_1$ plants, but segregation should be highly skewed to favor male or female genotypes. However, our results showed that those distorted co-dominant markers were skewed to favor heterozygotes and that the dominant markers did not show apparent favoritism for either the male or female genotypes (Table 1). Therefore, skewness in this study may not be caused by self-incompatible genes. It is interesting to note that markers located on LG1c were all skewed (Fig. 3) and that 1 locus skewed on 1 homoeologous chromosome may not indicate that its homoeolocus will be skewed on other chromosomes. For instance, both tf165c on LG1a and tf165BD on LG1c gave skewed segregation, but not tf165AE on LG1b (Fig. 3).

Each linkage group should ultimately correspond to one pair of chromosomes. Currently, well-characterized genetic stocks such as aneuploids, B-A translocations, and chromosome substitution lines are not available in tall fescue. Therefore, it is not possible to assign specific linkage groups to particular chromosomes or chromosome arms. A partial set of monosomic lines have been developed and analyzed by Dr. G. Eizenga at the USDA-ARS, University of Kentucky (personal communication). Monosomics would certainly be useful for assigning linkage groups to specific corresponding chromosomes.

Three maize probes detected segregation at 7 loci in tall fescue, of which 5 were on LG3a, LG3b, and LD3C, and 2 on homoeologous group 2. Thus, tall fescue homoeologous group-3 and -2 chromosomes may correspond to maize chromosome 1. Comparative mapping analysis has found gene synteny on many chromosome segments in maize, rice, sorghum, and wheat genomes (Ahn et al. 1994; Pereira et al. 1994). It has been proposed that the grass family may have originated from a common progenitor (Bennetzen and Freeling 1993). The maize genome is one of the most characterized crop species, and many of its functional genes have been identified, cloned, and mapped. Comparative mapping with maize probes (especially those functional cDNA probes) would provide not only information on the evolutionary divergence between tall fescue and

maize genomes but would also help identify the possible functions of particular chromosome regions in tall fescue.

Tall fescue is a perennial and can easily be asexually propagated from vegetative tillers. The parental lines (plants), $F_1$, and mapping population generated in this study are therefore permanent, and new RFLP mapping data can readily be added to the previous database. The map constructed in this study can be refined and used in future genetic studies and for the evaluation of quantitative traits in various environments for possible gene tagging.

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