A Demonstration of a 1:1 Correspondence Between Chiasma Frequency and Recombination Using a *Lolium perenne/Festuca pratensis* Substitution

J. King,*,† L. A. Roberts,† M. J. Kearsey,‡ H. M. Thomas,† R. N. Jones,* L. Huang,† I. P. Armstead,† W. G. Morgan† and I. P. King†,[1]

**Institute of Biological Sciences, University of Wales, Aberystwyth, SY23 3DA, Wales, United Kingdom, †Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23 3EB, Wales, United Kingdom and ‡School of Biosciences, University of Birmingham, Birmingham, B15 2TT, United Kingdom*

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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 ($2n = 2x = 14$). The chromatin of *F. pratensis* and *L. perenne* can be distinguished by genomic *in situ* hybridization (GISH), and it is therefore possible to visualize the substituted *F. pratensis* chromosome in the *L. perenne* background and to study chiasma formation in a single marked bivalent. Recombination occurs freely in the *F. pratensis/L. perenne* bivalent, and chiasma frequency counts give a predicted map length for this bivalent of 76 cM. The substituted *F. pratensis* chromosome was also mapped with 104 *Eco*RI/*Tru*91 and *Hin*dIII/*Tru*91 amplified fragment length polymorphisms (AFLPs), generating a marker map of 81 cM. This map length is almost identical to the map length of 76 cM predicted from the chiasma frequency data. The work demonstrates a 1:1 correspondence between chiasma frequency and recombination and, in addition, the absence of chromatid interference across the Festuca and Lolium centromeres.

THE generation of dense genetic linkage maps has been one of the major aims for geneticists studying large-genome plant and animal species, including humans. The ultimate goal of this mapping is to facilitate the location of genes responsible for the control of scientifically and agronomically important traits. DNA markers closely linked to a gene of interest are an important tool for indirect selection in plant and animal breeding programs and the identification of genes responsible for disease in humans (Lander and Schork 1994) and, in addition, provide a springboard from which genes can be isolated via map-based cloning strategies such as chromosome walking, chromosome landing, and chromosome jumping. However, maps generated using molecular markers often have greatly inflated lengths compared with those predicted from chiasma counts (Nilsson *et al.* 1993; Säll and Nilsson 1994; Sybenga 1996). This has raised key questions of fundamental importance regarding meiosis: What is the relationship between chiasma frequency and the size of genetic linkage maps? Are genetic maps too large or are chiasma counts too low? Alternatively, is there some mechanism other than chiasmata that is responsible for an appreciably high frequency of recombination? Moreover, the noncorrespondence of map length determined from chiasma counts and genetic mapping challenges one of the most fundamental principles of genetics; *i.e.*, recombination of linked alleles on homologous chromosomes is the result of crossing over and is visualized by chiasma formation.

It has been suggested that chiasma counts may be biased downward (Nilsson *et al.* 1993), as the number of chiasma appeared to decrease from diplotene to metaphase I. Darlington (1929) accounted for this observation with his theory of chiasma terminalization. However, a number of examples, using differentially stained chromosome analysis, have shown that the location of chiasmata and the sites of physical exchange coincide throughout the first meiotic division [*i.e.*, *Locusta migratoria* (Tease and Jones 1995), male mice (Kanda and Kato 1980), female mice (Polani *et al.* 1979), and male Armenian hamsters (Allen 1979)].

Given that terminalization appears not to exist, could low chiasma counts result from underscoring? First, it might not be possible to distinguish between a single chiasma and two closely adjacent chiasmata (Sybenga 1975). Second, Nilsson *et al.* (1993) suggested closely spaced chiasmata may cancel each other out so as not to form a chiasma at all. However, this suggestion based on data from Tease and Jones (1978) was later refuted by Tease and Jones (1995).

An alternative hypothesis to explain genetic maps longer than those predicted from chiasma counts is that a mechanism other than chiasmata is also responsible for recombination. If the frequency of recombination generated by such a mechanism was sufficiently high it might explain the discrepancy between chiasma and recombination frequency observed between cytological

[1]*Corresponding author:* IGER, Plas Gogerddan, Aberystwyth, SY23 3EB, Wales, United Kingdom. E-mail: ian.king@bbsrc.ac.uk

and mapping analysis. Thus Gill *et al.* (1995) observed exchanges of short interstitial segments that were not recovered as chiasmata in interspecific wheat hybrids and similar observations, in interspecific hybrids of rice, were made by Jena *et al.* (1992) and Ishi *et al.* (1994). Gene conversion, involving a nonreciprocal exchange of DNA, will also lead to recombination without the formation of chiasmata. Both of these possibilities would lead to an apparent increase in the number of crossovers required to explain genetic map lengths while leaving chiasma counts unaffected.

The question remains, Are the recombinant maps too large? A considerable weight of evidence indicates that the answer to this question may be yes. Many genetic linkage maps have been produced using the Kosambi mapping function (Kosambi 1944), which assumes that interference between chiasmata decreases linearly with recombination frequencies (Kosambi 1944). Thus map inflation will occur because the Kosambi function does not allow for variation in interference or crossover localization (Sybenga 1996). In addition, different mapping programs give rise to different levels of map inflation. MAPMAKER (Lander *et al.* 1987) and JOINMAP (Stam 1993) are commonly used programs for generating genetic maps. Of the two programs MAPMAKER produces the most inflated maps (Lincoln and Lander 1992; Van Ooijen *et al.* 1994; Castiglioni *et al.* 1998).

Map inflation may also occur as a result of misclassification of molecular markers. Indeed Lincoln and Lander (1992) concluded that just 5% classification errors could increase map length by 50%. In humans it has been demonstrated that singletons (tight linked double recombinants) are generally the result of genotyping errors (Buetow 1991; Tomfohrde *et al.* 1992; Zahn and Kwiatkowski 1995). Broman *et al.* (1998) showed that singletons resulted in an increase in map length of ~25% in both male and female maps. A further consideration is that in many of the examples in the literature where the lengths of genetic maps produced using molecular markers are compared to the lengths estimated using chiasma counts, the two sets of data have not been generated from the same material. Genetic background, heterosis, and sex differences can all have profound effects on chiasma and recombination frequencies (Hultén 1994; Van Ooijen *et al.* 1994; Sherman and Stack 1995; Sybenga 1996).

In this article we have cytologically analyzed and genetically mapped a *Festuca pratensis* ($2n = 2x = 14$) chromosome in a *Lolium perenne* ($2n = 2x = 14$)/*F. pratensis* monosomic substitution line (*i.e.*, 13 *L. perenne* chromosomes + 1 *F. pratensis* chromosome). *L. perenne/F. pratensis* monosomic substitutions are very unusual because even though the *F. pratensis* chromosome and its *L. perenne* homeologue recombine at high frequency they can easily be distinguished using genomic *in situ* hybridization (GISH; King *et al.* 1998, 1999). The ability to distinguish the *F. pratensis* chromosome in the *L. perenne/F. pratensis* monosomic substitution line at meiosis using GISH has allowed us to study the frequency of chiasmata in the *L. perenne/F. pratensis* bivalent. These cytological observations have been combined with data from a dense amplified fragment length polymorphism (AFLP) map of the *F. pratensis* chromosome to answer the fundamental question, Is there a 1:1 correspondence between chiasma frequency and recombination?

MATERIALS AND METHODS

The 14-chromosome *L. perenne/F. pratensis* monosomic substitution [BC_1 57, which carries a nucleolar organizer region (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). In a single season anthers were harvested from BC_1 57 for meiotic analysis and 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 backcross individuals were randomly chosen for AFLP mapping.

***In situ* hybridization:** GISH (using *F. pratensis* genomic DNA as a probe) and fluorescent *in situ* hybridization [FISH; using 18S-26S rDNA (Gerlach and Bedbrook 1979) as probe, *i.e.*, pTa 71] analysis of pollen mother cell (PMC) preparations of the monosomic substitution 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.

Cytological mapping: The predicted genetic length of the Festuca chromosome was calculated from the mean number of chiasmata scored in the *F. pratensis/L. perenne* bivalent (μ), visualized with GISH, using the expression 50 μcM; *e.g.*, a chromosome with an average of two chiasmata is expected to have a genetic length of $50 \times 2 = 100$ cM (Kearsey and Pooni 1996).

AFLP mapping: AFLP analysis was carried out as described by King *et al.* (1998) with the exception that AFLPs were visualized using the Silver Sequence DNA sequencing system (Promega, Madison, WI). Two enzyme combinations were used: (i) *Eco*RI/*Tru*91 and (ii) *Hin*dIII/*Tru*91. Primers with one additional nucleotide were used for the preamplification to maximize primer selectivity. The additional nucleotide used on both primers was adenine. Primers for the selective amplification all had three additional nucleotides. *Tru*91 was used instead of its isoschizomer *Mse*1. Therefore the primers used for *Tru*91 were those described for *Mse*1 by Vos *et al.* (1995), and the original labeling of "M" as a prefix for these primers has been retained. The initial AFLP analysis was carried out to find markers specific to the Festuca chromosome in BC_1 57. To do this the DNA from the four plants involved in the production of BC_1 57, *i.e.*, *F. pratensis*, *L. perenne* (tetraploid), *L. perenne* (diploid), and the Lolium/Lolium/Festuca triploid hybrid, as well as BC_1 57 itself, were screened. Markers found to be present in *F. pratensis*, the triploid hybrid, and BC_1 57, but absent from *L. perenne* (both the diploid and tetraploid genotypes), were classified as being specific to the BC_1 57 Festuca chromosome. Screening was carried out with all possible combinations of the *Eco*RI primers with all the *Tru*91 primers and also with all possible combinations of the *Hin*dIII primers with the *Tru*91 primers. Primer combinations to screen the BC_2 plants were then chosen on the basis of (a)

the number of multiple markers and (b) marker clarity. The software package JOINMAP 2.0 (Stam 1993) was used to generate a genetic linkage map from the AFLP marker data. The genetic map produced by JOINMAP also allowed the presence or absence of the *F. pratensis*-specific AFLP markers to be examined in each of the BC_2 plants.

RESULTS

The genetic map: A total of 512 primer combinations were used to screen the parental genotypes, resulting in the identification of >400 possible markers. Sixteen *Eco*RI/*Tru*91 primer combinations and 19 *Hin*dIII/*Tru*91 primer combinations were selected, giving 50 and 54 polymorphic markers, respectively, *i.e.*, H38/M43, E34/M35 (1 polymorphism); E33/M38, E38/M32, H31/M40, H32/M37, H33/M33, H33/M38, H35/M41, H36/M33, H38/M36, H39/M37, H41/M33 (2 polymorphisms); E32/M41, E33/M41, E34/M33, E39/M38, E40/M40, H35/M42, H36/M36, H36/M37, H37/M33, H37/M36 (3 polymorphisms); E34/M32, E36/M43, E36/M46, E38/M40, E39/M32, H42/M35 (4 polymorphisms); E32/M40, H35/M39, H44/M39 (5 polymorphisms); E36/M37, H43/M40 (6 polymorphisms); and E33/M37 (7 polymorphisms). These 35 primer combinations were used to screen the 148 randomly chosen plants making up the BC_2 mapping population. Markers were scored simply for presence or absence of a band. An initial genetic linkage map of the Festuca chromosome of 91 cM was generated using JOINMAP 2.0 (Stam 1993). To calculate map distances the analyses were performed using a LOD threshold of 3 and Kosambi's mapping function (Kosambi 1944). To keep scoring errors to a minimum all AFLP primer pairs/genotypes giving rise to singletons and questionable markers (as defined by the mapping program) were rerun and reanalyzed at least once. This gave rise to a final genetic map of 81 cM (Figure 1).

The centromere is physically located 49.2% along the chromosome from the telomere of the arm that lacks the NOR while its genetic position is at 59.2 cM, *i.e.*, 73% along the genetic map (King *et al.* 2002, accompanying article). A comparison of the physical and genetic position of the centromere revealed that with three exceptions only one recombinant event occurred per chromosome arm in the *F. pratensis/L. perenne* bivalent, *i.e.*, a maximum of two recombinant events per bivalent. In the three exceptions two recombinant events had occurred in the arm without the NOR.

The AFLP markers appeared to give good genome coverage, although the 104 markers mapped to only 43 separate positions on the genetic map, indicating considerable clustering. The largest gap between markers was only 5.9 cM on the chromosome arm without the NOR. The two different types of AFLP did not show quite the same pattern of clustering. *Hin*dIII/*Tru*91 markers were much more evenly spread, with two "modest" clusters at 30–40 cM and 59–70 cM. These two clusters contained 48% of the *Hin*dIII/*Tru*91 markers in 26% of the genetic length of the chromosome. The *Eco*RI/*Tru*91 AFLP markers showed one very large cluster that coincided with the second *Hin*dIII/*Tru*91 cluster at ~59–70 cM. This segment represents only 14% of the genetic map but contained 62% of the *Eco*RI/

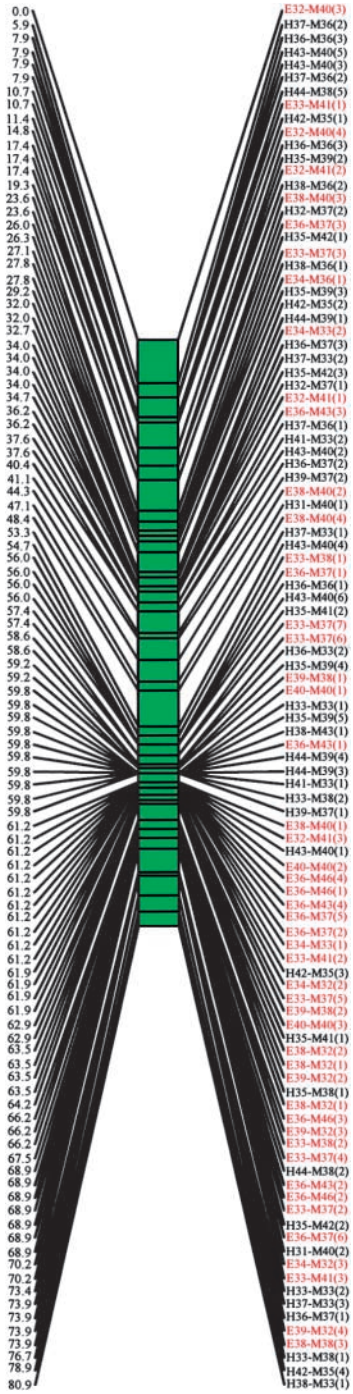

Figure 1.—The genetic map of the *Festuca pratensis* chromosome in the monosomic substitution BC_1 57. The black numerals on the left of the chromosome indicate the genetic distance in centimorgans. The red and black text on the right indicates the position of the *Eco*RI/*Tru*91 and *Hin*dIII/*Tru*91 Festuca-specific polymorphisms.

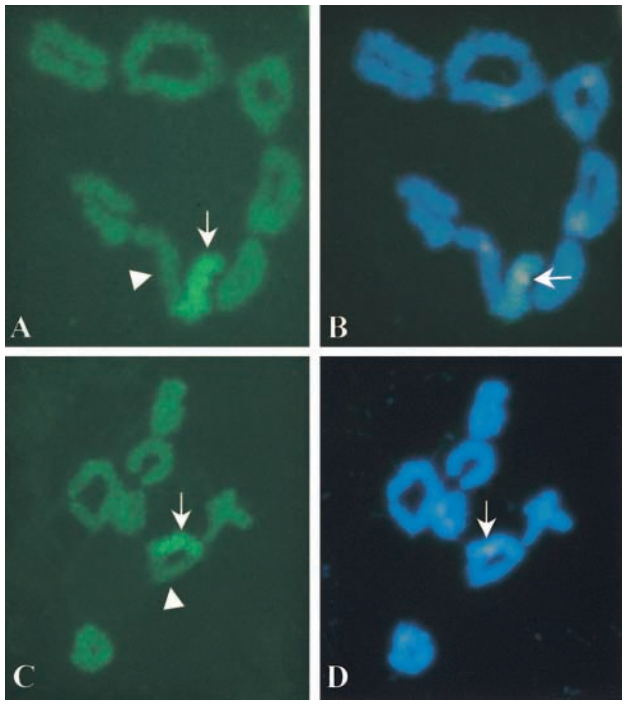

FIGURE 2.—GISH and FISH analysis of BC_1 57 PMCs at diakinesis. (A and C) GISH images using total genomic Festuca DNA as probe (*i.e.*, digoxigenin conjugated to FITC); the Festuca chromosome is bright green (arrows) while the homeologous Lolium chromosome is dull green (arrowheads). (A) A homeologous Festuca/Lolium rod bivalent with a single chiasma. (C) A homeologous Festuca/Lolium ring bivalent with a single chiasma in each arm. (B and D) FISH images using pTa 71 as probe (Cy-3) of the same cells. The pTa 71 sites on the Festuca chromosomes are indicated by arrows (the remaining sites of pTa 71 hybridization are located on Lolium chromosomes).

*Tru*91 AFLPs. The region of the genetic map from 60 to 70 cM also contains both the centromere and the NOR (KING *et al.* 2002, accompanying article).

Estimated length of the genetic map: GISH applied to anther squashes of the monosomic substitution line BC_1 57 allowed the frequency of chiasma formation between the *F. pratensis* chromosome and its *L. perenne* homeologue to be recorded at diakinesis in 228 PMCs (Figure 2). Of the 228 PMCs analyzed 109 contained rod bivalents while 119 contained ring bivalents (no more than 2 chiasmata were observed in any of the *F. pratensis/L. perenne* bivalents). The average chiasma frequency for the bivalent was 1.522 on the basis of 347 chiasmata in 228 PMCs. This value provided an estimate of the genetic length of the *F. pratensis* chromosome using the expression 50 μcM, *i.e.*, $50 \times 1.522 = 76$ cM, which is very similar to the length of 81 cM obtained by mapping of the AFLP markers.

A direct statistical comparison of the genetic map lengths generated from the chiasma counts and AFLP data was not possible due to the map lengths being based on percentage of recombination frequencies. It was therefore necessary to compare the expected number of exchanges in the BC_2 mapping population predicted by counting chiasmata and the number of observed exchanges in the BC_2 population detected by the AFLP markers.

It is possible to predict the type and frequency of recombinant or nonrecombinant chromatids (and hence the number of exchanges) generated, when either one or two chiasmata are formed within the *F. pratensis/L. perenne* bivalent in BC_1 57 at meiosis, assuming that the ratio of rods (one-chiasmate bivalents) to rings (bivalents with at least one chiasma in each arm) is known and that there is no chromatid interference (KEARSEY and POONI 1996). Bivalents involving two chiasmata give rise to types and proportions of recombinant chromatids different from those involving one chiasma. Bivalents with one chiasma will always give rise to 50% recombinant and 50% nonrecombinant chromosomes (KEARSEY and POONI 1996). In the absence of chromatid interference the position of the second chiasma in bivalents with two chiasmata is expected to be independent of the first in terms of the chromatids involved. Thus 50% of two-chiasmate bivalents will involve three-strand exchanges while the remaining 50% will be composed of equal numbers of two- and four-strand exchanges. Overall, two-chiasmate bivalents will therefore be expected to give rise to 25% nonrecombinants, 50% single recombinants, and 25% double recombinants (KEARSEY and POONI 1996).

The GISH analysis of BC_1 57 PMCs showed that the proportion of rod bivalents to ring bivalents formed between the Festuca chromosome and its homeologue was not significantly different from a 1:1 ratio (*i.e.*, $\chi^2_{[1]} = 0.44$ NS). Rods were found in 48% of PMCs and rings in 52%. No univalents and no higher-order configurations were seen. The expected frequencies of non-, single-, and double-recombinant chromosomes in the BC_2 population were therefore calculated using this observed ratio of rods to rings. For example, of the 48% observed rods, 50% would be nonrecombinants and 50% would be single recombinants (KEARSEY and POONI 1996). Thus the BC_2 population would be expected to contain 24% nonrecombinants and 24% single recombinants generated by single chiasmate bivalents. The expected frequencies were used to predict the numbers of each type of chromosome that would be found in the BC_2 population used, *i.e.*, 148 plants (Table 1).

The actual numbers of non-, single-, and double-recombinant chromatids were determined from the 148 individuals in the BC_2 mapping population using the presence or absence and order of AFLP markers. Observed numbers of each type of chromosome are shown in Table 1. A χ^2 test ($\chi^2_{[2]} = 0.53$) on the observed and expected numbers of nonrecombinant and single- and double-recombinant chromosomes proved not significant at the 5% level of probability, showing that the AFLP data were consistent with the chiasma counts.

TABLE 1

The frequency of recombinant chromosomes in the BC_2 Population

		Single recombinants		Double recombinants	
	Non recombinants	Non-NOR arm	NOR arm	One per arm	Two in non-NOR arm
Observed nos.	59	53	18	15	3
		71		18	
Expected nos. (no chromatid interference)	54.76	52.8	21.2	19.24	0
		74		19.24	
Expected nos. (chromatid interference)	35.5	112.7		0	

Shown are the observed numbers of nonrecombinant and recombinant chromosomes in the BC_2 population of 148 plants and the arm locations of the recombination site. Also shown are the expected numbers of nonrecombinant and recombinant chromosomes with a BC_2 population of 148 plants based on the observed frequencies of rod and ring bivalents without and with complete chromatid interference. An example of expected numbers calculation for single recombinants (no chromatid interference) is as follows:

1. Rods:rings, 48%:52%.
2. Rods = 50% nonrecombinants and 50% single recombinants (see Figure 1). Therefore in total population 24% = single recombinants.
3. Rings = 25% nonrecombinants, 50% single recombinants and 25% double recombinants (see Figure 1). Therefore in total population 26% = single recombinants.
4. 24% + 26% = 50% single recombinants = 74 plants out of the 148.

In addition, the *F. pratensis* chromosome carried a cytological marker, *i.e.*, the NOR, which was visualized by FISH using the pTa 71 probe in combination with GISH (Figure 2). Thus it was possible to determine which arm or arms of the chromosome were involved in chiasma formation. Analysis of the chiasma frequency between the *F. pratensis* chromosome and its *L. perenne* homeologue revealed that of the 109 rods the *F. pratensis* arm carrying the NOR was the unpaired arm on all but six occasions. The ability to determine chiasma frequency for the individual arms also allowed the estimation of genetic distance of each arm. Thus in the 228 PMCs observed, the non-NOR arm had 222 chiasmata (103 rods + 119 rings) while the NOR arm had 125 (6 rods + 119 rings), giving mean chiasma frequencies of 0.974 and 0.548, respectively. The arm carrying the NOR was therefore estimated to be 27.4 cM long (*i.e.*, 50 × 0.548) while the other arm was estimated to be 48.7 cM long (*i.e.*, 50 × 0.974). The corresponding genetic distances (as calculated by JOINMAP using the AFLP marker data) were found to be 20.9 and 60 cM, respectively.

It was again necessary to statistically compare the map lengths indirectly; *i.e.*, the number of observed crossover events for each arm as determined from the AFLP data was compared to the number of crossover events expected from the chiasma counts (Table 1).

The expected numbers of crossovers per arm were derived as follows. A total of 48% of the configurations observed were rods. Thus in the BC_2 population one-half, *i.e.*, 24%, would be expected to yield nonrecombinants and 24% would be expected to yield single recombinants. However, 103 of the 109 (94.5%) rods observed involved recombination in the non-NOR arm while 6 of the 109 rods (5.5%) involved recombination in the NOR arm. Therefore, in the BC_2 population the rods would yield 24% nonrecombinants, 22.7% single recombinants with the recombination site in the non-NOR arm, and 1.3% single recombinants with the recombination site in the NOR arm. The remaining 52% of configurations observed were ring bivalents with one chiasma per chromosome arm. In the BC_2 population these would be expected to yield 13% nonrecombinants, 26% single recombinants, and 13% double recombinants. Of the 26% single recombinants it was assumed that the recombination site occurred at an equal frequency in the two arms, *i.e.*, 13% in the non-NOR arm and 13% in the NOR arm. The expected frequencies from ring bivalents were added to the expected frequencies from rod bivalents and the totals were used to predict the numbers of non-, single-, and double-recombinant chromosomes and the arm in which the recombination sites were located in the BC_2 mapping population of 148 plants (Table 1).

The expected and observed numbers of non-, single-, and double-recombinant chromosomes and the arm location of the recombination site(s) given in Table 1 were used to calculate the number of exchanges in each of the individual arms of the Festuca chromosome (Table 2).

The chi-square test was then used to compare the expected number of exchanges and the observed num-

TABLE 2

The frequency of crossovers in each Festuca chromosome arm

Arm	Non-NOR			NOR		
No. of crossovers	0	1	2	0	1	2
Observed nos.	77	68	3	115	33	0
Expected nos.	75.96	71.94	0	107.46	40.34	0

Shown is the frequency of observed and expected number of crossovers in each arm of the Festuca chromosome in 148 BC_2 plants based on the data presented in Table 1.

ber of exchanges in each arm. The test was carried out on data for no exchanges and data for one or more exchanges. This was because although not more than one chiasma per chromosome arm was observed in the 228 PMCs analyzed using GISH, the AFLP data did reveal three plants with two recombination sites in the non-NOR arm. Both tests ($\chi^2_{[1]} = 0.026$ and $\chi^2_{[1]} = 1.865$) were not significant at the 5% level of probability.

The occurrence of chromatid interference would have caused the nonrandom involvement of chromatids in consecutive chiasmata (described by Kearsey and Pooni 1996), thereby altering the predicted frequencies of non-, single-, and double-recombinant chromosomes, *i.e.*, leading to a significant deviation from a 1:1 correspondence between chiasma frequency and recombination. Complete chromatid interference would lead to two consecutive chiasmata involving only four different chromatids; *i.e.*, two-strand and three-strand crossovers would be eliminated. Table 1 therefore shows the expected numbers of non-, single-, and double-recombinant chromosomes in a population of 148 plants, in the presence of chromatid interference (four-strand exchanges only). A chi-square analysis to compare the expected numbers of the non-, single, and double recombinants in the presence of chromatid interference with the observed numbers of these three classes was highly significant ($\chi^2_{[1]} = 20.7$), thus demonstrating the absence of complete chromatid interference across the Festuca and Lolium centromeres.

DISCUSSION

The research described demonstrates that *L. perenne/F. pratensis* hybrids and introgression lines provide an invaluable method for studying genetic recombination and generating chromosome-specific genetic maps. As a result of the high level of DNA polymorphism between *L. perenne* and *F. pratensis* it was possible to quickly isolate a large number of *F. pratensis*-specific AFLPs from which a genetic map was constructed. In addition, the ability to distinguish the *F. pratensis* chromosomes from *L. perenne* chromosomes at meiosis, using GISH, made it possible to determine the chiasma frequency in the *F. pratensis/L. perenne* bivalent in the monosomic substitution BC_1 57, the genotype that gave rise to the BC_2 mapping population. This allowed the expected genetic length of the *F. pratensis* chromosome, on the basis of visual observation, to be compared with the actual genetic distance determined by *F. pratensis*-specific AFLP markers.

The genetic linkage map of 81 cM constructed from the AFLP markers was not significantly different from that predicted by chiasma frequencies, *i.e.*, 76 cM, observed at diakinesis in 228 PMCs. This result is in contrast to many reports (Nilsson *et al.* 1993), which have shown large discrepancies between estimated genetic distances based on chiasma frequencies and genetic distances based on the segregation of genetic markers. Nilsson *et al.* (1993) and Sybenga (1996) put forward several possible explanations to account for the discrepancies in the lengths of genetic maps generated through molecular markers and those estimated by chiasma frequency:

1. Chiasma counts too low
   a. two adjacent chiasmata scored as single chiasma or not counted at all (Sybenga 1996)
   b. two adjacent chiasmata canceling each other out (Nilsson *et al.* 1993)
   c. recombination not involving chiasma, *e.g.*, gene conversion.
2. Genetic maps too long
   a. inaccurate scoring of molecular markers
   b. incorrect application of the mapping function.

Underscoring of chiasma could contribute to the genetic length discrepancies in some species, *e.g.*, those with a recombination rate higher than that found in the *L. perenne/F. pratensis* introgression lines, such as the *Gasteria lutzii* × *Aloe aristata* hybrid (Takahashi *et al.* 1997), Alstroemeria (Kamstra *et al.* 1999), and Allium (Khrustaleva and Kik 2000). In these examples GISH revealed a frequency of recombination in progeny derived from the respective interspecific hybrids much higher than that expected from the observed chiasma frequency in the parental genotypes. The chiasmata corresponding to the multiple crossovers in narrow chromosome segments seen in these examples are thus difficult to distinguish and could therefore be underscored (Takahashi *et al.* 1997; Kamstra *et al.* 1999; Khrustaleva and Kik 2000).

In this study no evidence indicated that chiasmata were being underscored, *e.g.*, multiple chiasmata in close proximity mistakenly scored as a single chiasma. GISH analysis of 16 BC_2 genotypes derived from BC_1 57 showed no evidence of multiple recombination sites on a single chromosome arm (J. King and I. P. King, unpublished data) as was the case in the *G. lutzii* × *A. aristata* hybrid (Takahashi *et al.* 1997), Alstroemeria (Kamstra *et al.* 1999), and Allium (Khrustaleva and Kik 2000). In addition, the AFLP marker data showed clearly the very low frequency with which two chiasmata occurred in the same chromosome arm, *i.e.*, 2%. The

fact that each chiasma observed was a single recombination event and that the predicted genetic length of the *F. pratensis* chromosome was almost identical to the genetic length determined by the AFLP markers are entirely consistent with a 1:1 ratio of chiasmata to recombination in the *F. pratensis/L. perenne* bivalent in BC_1 57.

The work in this article describes recombination between homeologous chromosomes of two different species, rather than recombination between homologous chromosomes within a species, and the question can be raised as to whether the relationship between chiasmata and genetic recombination is the same in both situations. We argue that it is only through the uniqueness of our experimental system, and use of a monosomic addition line, that we have been able to test this relationship with this degree of stringency. The mapping population that we used included *L. perenne* and *F. pratensis* genotypes as well as the hybrid material, and the AFLPs in the two parental lines have the majority of their bands in common with the monosomic substitution line, suggesting a common basis of genetic organization. The mapping of the Festuca chromosome proceeded as expected, and in addition the genetic order of markers was confirmed by physical mapping (King *et al.* 2002, accompanying article). The 1:1 correspondence between chiasma frequency and recombination has been demonstrated, albeit in a unique genotype, and there is no reason to believe that the same relationship does not hold in the same way as within a "pure species." Had we established this relationship within a species it could be just as strongly argued that this would also represent a unique situation without general applicability.

These results correspond to those of Tease and Jones (1995) whose work on differentially stained chromosomes of grasshoppers also gave a 1:1 correspondence of recombination and crossover sites. This work is very rarely quoted in research that shows unexpectedly inflated map lengths compared to that expected from chiasma frequency, because it places emphasis on experimental error, *i.e.*, inaccurate scoring of data. Further work supporting a 1:1 correspondence is seen in the work on human males where the expected genetic length of the male map based on chiasma counts, *i.e.*, 2605 cM (Hultén 1974), is very close to the actual map length obtained using molecular markers, *i.e.*, 2700 cM (Broman *et al.* 1998), even though the materials under investigation in the two reports were of different origin.

A possible cause for map inflation is the misclassification of markers (Lincoln and Lander 1992). In this work the initial genetic length of the Festuca chromosome was 90.5 cM. However, when AFLP primer pairs giving rise to questionable markers, *i.e.*, singletons, were rerun, and the data scored, the genetic length of the Festuca chromosome was reduced to 81 cM. Thus the misclassification of markers led to map inflation. This is in agreement with human genome mapping, which has shown that the removal of erroneous singletons causes large reductions in map length (Dib *et al.* 1996; Broman *et al.* 1998). In the present work correcting individual data points often had a considerable effect on the JOINMAP output, sometimes causing rearrangements to the order of genetic markers in positions several centimorgans away from the AFLP marker actually altered. Such changes are a common feature of mapping with dense marker maps and reflect the inherent uncertainty of the true map order.

As a result of the 1:1 correspondence between chiasma and recombination there was no need to invoke any other mechanism, *e.g.*, gene conversion, in this work. This does not, however, rule out the possibility that gene conversion did occur at a very low but undetectable frequency. Gene conversion events have been shown to occur in humans (Jeffreys *et al.* 1994) but are thought to be too rare to have a major effect on map lengths (Broman *et al.* 1998). It can therefore be concluded that crossing over, visualized as chiasmata, is the primary mechanism by which recombination occurs between linked alleles on homologous chromosomes.

It is important to note that in the reports that show discrepancies between predicted map lengths obtained from chiasma counts and map lengths obtained from genetic mapping with molecular markers, the two types of analysis were not performed on the same material under the same conditions. Hultén (1994), Sherman and Stack (1995), and Sybenga (1996) point out that different genetic backgrounds, heterosis, and sex differences have all been shown to cause variation in recombination frequencies. The work described here is the only example where the same material has been used concurrently for meiotic analysis and the generation of a BC_2 mapping population.

The results reported here failed to demonstrate any evidence for chromatid interference and therefore do not agree with the previous reports that suggested chromatid interference to be occurring (Darlington and Dark 1932; Hearne and Huskins 1935; Huskins and Newcombe 1941). They are, however, in agreement with the previous research of Jones and Tease (1979), who were able to trace individual chromatids through meiosis I using differentially stained chromosomes of grasshoppers, and Brown and Zohary's (1955) work on *Lilium formosanum.* However, in all but a few cases *F. pratensis/L. perenne* bivalents carried a single chiasma per chromosome arm. Thus it was not possible to determine if chromatid interference did or did not operate between adjacent chiasmata in the same chromosome arm.

In conclusion, because there was generally only one chiasma per chromosome arm, it is possible to demonstrate that there is a 1:1 correspondence between chiasma frequency and recombination. The large discrepancies between chiasma frequency and recombination in other work are probably due to (1) underscoring of chiasmata, which leads to an underestimate of map

length; (2) mapping inaccuracy (misscoring, inaccurate mapping programs), which results in map lengths larger than expected; and/or (3) meiotic analysis and genetic mapping being carried out on different material under different conditions.

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