Cytogenetic and Genome Research

Cytogenet Genome Res
DOI: 10.1159/000313379

Published online: May 26, 2010

Physical Distribution of Homoeologous Recombination in Individual Chromosomes of *Festuca pratensis* in *Lolium multiflorum*

D. Kopecký[a] M. Havránková[a] J. Loureiro[b] S. Castro[b] A.J. Lukaszewski[c]
J. Bartoš[a] J. Kopecká[d] J. Doležel[a]

[a]Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Olomouc, Czech Republic; [b]Centre for Functional Ecology, Department of Life Sciences, Faculty of Science and Technology, University of Coimbra, Coimbra, Portugal; [c]Department of Botany and Plant Sciences, University of California, Riverside, Calif., USA; [d]Department of Biology, Pedagogical Faculty, Palacky University, Olomouc, Czech Republic

Key Words
Chromosome introgression · *Festuca* · Flow cytometry · Genome size estimation · Homoeologous recombination · Karyotype · *Lolium*

Abstract
Crossing over-based recombination is a powerful tool for generating new allelic combinations during sexual reproduction. It usually occurs between homologous chromosomes. However, under some conditions, homoeologues may also be capable of crossing over. Whether homologous and homoeologous crossovers are equivalent and governed by the same rules has never been established. Here we report on chromosome distribution of homoeologous crossovers in a unique system of *Festuca* × *Lolium* hybrids. Unlike in most other hybrids, in these intergeneric hybrids, homoeologous chromosomes are capable of pairing and crossing over with frequencies approaching that of homologues. At the same time, genome divergence makes cytological detection of chromosome recombination feasible. We analyzed the distribution of homoeologous recombination along individual chromosomes in a complete set of intergeneric single chromosome substitutions from *F. pratensis* into tetraploid *L. multiflorum*. Homoeologous recombination sites were not evenly distributed along the chromosomes, being concentrated in intercalary regions of the arms and reduced in proximal and distal regions. Several recombination hotspots and cold spots were found along individual chromosomes and the recombination was not affected by the presence of a secondary constriction. Our results indicate that despite the uneven distribution of homoeologous recombination, introgression of any part of the *F. pratensis* genome into *L. multiflorum* is feasible.

Perennial ryegrass (*Lolium perenne* L.) and Italian ryegrass (*L. multiflorum* Lam.) are among the most agriculturally important grass species in the temperate regions. They are widely used for forage and turf. Both species are cultivated in monocultures as well as in mixtures with other species [Fojtík, 1994; Černoch et al., 2003]. Possible changes in the global climate call for broadening of the gene pool of both species as a buffer against potentially harsher growing conditions. Such broadening of the gene

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1424–8581/10/0000–0000$26.00/0

Accessible online at:
www.karger.com/cgr

D. Kopecký
Institute of Experimental Botany
Sokolovská 6
CZ–77200 Olomouc (Czech Republic)
Tel. +420 585 205 857, Fax +420 585 205 853, E-Mail kopecky@ueb.cas.cz

pool can be achieved by intra- and interspecific hybridization. Because of the generally poor survival rate of both ryegrass species under abiotic stress conditions, the latter strategy appears to be more appropriate [Jauhar, 1993]. Ideal donor species of tolerance to abiotic stresses belong to *Festuca* subgen. *Schedonorus*. Species of this subgenus are closely related to ryegrasses [Catalán et al., 2004], and especially *F. arundinacea* Schreb. and *F. pratensis* Huds. exhibit high tolerance to abiotic stresses, such as drought and freezing [Jauhar, 1993].

The potential of interspecific hybridization for gene transfer via introgression breeding is generally limited by the inability of parental chromosomes to pair and recombine. This is true also in allopolyploid species, which often possess genetic systems preventing homoeologous pairing such as *Ph1* genes in wheat [Riley and Chapman, 1958; Sears and Okamoto, 1958] and *PrBn* in *Brassica napus* [Jenczewski et al., 2003]. However, *Festuca* and *Lolium* species are unique, because their chromosomes pair with each other and recombine freely [Thomas et al., 1994; Kopecký et al., 2008, 2009a].

There are 2 strategies of interspecific breeding. One is the development of amphiploids. A good example of the use of man-made amphiploids is hexaploid triticale (×*Triticosecale* Wittmack), a wheat-rye hybrid. Perhaps the main reason why this amphiploid is agronomically successful is that both parents are cultivated species. When one of the parent species is undomesticated, it tends to contribute to the amphiploid not only desirable but also weedy characteristics and these may require considerable long-term breeding efforts to mitigate. Hence the introgression breeding represents a strategy to introgress only the desirable characteristics into a cultivated species. In this approach, the initial cross is followed by recurrent backcrosses to the recipient species, during which the introgressed region is narrowed down, eliminating the undesired genetic ballast while retaining the specific traits of interest. Clearly, the success of this strategy is dependent on the frequency and distribution of recombination across parental genomes and it is known that the distribution of crossovers along the chromosomes is not random.

In large genomes, such as wheat and barley, the frequency of crossovers increases from centromeres to telomeres [Lukaszewski and Curtis, 1993; Künzel et al., 2000]. However, the increase is not gradual and crossovers preferentially occur in certain regions of chromosomes called 'recombination hotspots'. In contrast to hotspots, there are regions where almost no crossovers occur – the 'recombination cold spots' [Mézard, 2006]. The total number of hotspots in humans was estimated at ~50,000 [Buard and de Massy, 2007]. Meiotic recombination hotspots have also been identified in several plant species, such as *Arabidopsis thaliana* [Mézard, 2006], rice [Kurata et al., 1994], wheat [Gill et al., 1996] and *Lolium/Festuca* [King et al., 2002]. In barley, hotspots were preferentially localized to distal regions of chromosomes, but they were also found in interstitial positions [Künzel et al., 2000]. In *L. perenne*/*F. pratensis* introgression lines, the hotspots were also localized in the distal regions [King et al., 2002], but not in the most distal 10% of the physical chromosome length as in barley [Künzel et al., 2000].

Species-specific distribution of recombination hotspots was described by Winckler et al. [2005]. The authors compared humans and chimpanzees and found that only 8% of recombination hotspots were common to both species. Interestingly, the distribution of recombination hotspots can change during evolution. Several sites with high recombination rates in humans show average or low recombination rates in chimpanzee, and vice versa [Jeffreys et al., 2005]. The size of hotspots can be relatively small; for example in human sperm cells, hotspots of ~2 kb length were identified [Buard and de Massy, 2007]. In plants, regions of extremely high recombination rates may represent only a few kilobases [Dooner and Martinez-Ferez, 1997; Okagaki and Weil, 1997].

The frequency and distribution of recombination events is influenced by intrinsic and extrinsic factors, such as sex [Lenormand and Dutheil, 2005], temperature [Francis et al., 2007], chemical agents and physical stress [Sinha and Helgason, 1969]. Differences in crossover rates in specific regions of the genome were detected among cultivars of many crop species, including barley, suggesting the effect of the genotype [Sall et al., 1990]. Crossover frequency can also be changed by chromosome rearrangements. Thus, even a short terminal deletion of a chromosome arm in *L. perenne* significantly reduced the frequency of crossovers [Jones ES et al., 2002]. In wheat and in rye, crossover distribution can be manipulated by structural aberrations [Sybenga, 1975; Jones LE et al., 2002; Qi et al., 2002], dramatically increasing the crossover saturation rate in designated chromosome segments when they are placed in terminal positions. On the other hand, a whole-arm inversion in rye inverted the pattern of chiasma distribution suggesting that the typical pattern of recombination in cereals, with its concentration at the telomere and absence at the centromere may in fact reflect an innate ability of specific chromosome regions to form crossovers, along the line of the zygomere

concept of Sybenga [1969] and 'pairing centers' of Maguire [1986].

As mentioned earlier, the possibility to introgress just one or a few attractive traits from one species to another (usually an agronomically important crop) is of great importance. For this, an association between the distribution of recombination events and the location of gene-rich regions plays a key role in the success or failure of introgression breeding. In wheat and maize, a high correlation was observed between recombination frequency and the distribution of gene-rich regions [Akhunov et al., 2003a, b; Shah and Hassan, 2005; Anderson et al., 2006]. Similarly in barley, the distribution of recombination hotspots was positively correlated with that of DNA markers assumed to be gene-derived [Künzel et al., 2000]. However, such examples may not represent a general rule. In *Arabidopsis*, most of the recombination events in hotspots were localized to non-coding regions [Drouaud et al., 2006; Mézard, 2006]. Similarly, King et al. [2007] found that most of the functionally annotated genes were located in regions with low recombination rates in *Lolium*/*Festuca* introgression lines.

In this study we performed a detailed analysis of the distribution of homoeologous crossovers at the chromosomal level in *L. multiflorum*/*F. pratensis* substitution lines. As the first step, we established the karyotypes, estimated nuclear genome sizes and determined molecular chromosome sizes in both parental species. Subsequently, we compared the relative lengths of individual homoeologous chromosomes and measured the positions of homoeologous recombination events in each of the 7 chromosomes of *F. pratensis* in *L. multiflorum*/*F. pratensis* substitution lines.

Material and Methods

Plant Material

Nuclear DNA content was estimated in diploid cultivar 'Kolumbus' (2n = 2x = 14) and tetraploid cultivar 'Patra' (2n = 4x = 28) of *F. pratensis*, and in diploid cultivar 'Prolog' (2n = 2x = 14) and tetraploid cultivar 'Lubina' (2n = 4x = 28) of *L. multiflorum*. Moreover, we also estimated nuclear DNA amounts in diploid cultivar 'Handicap' (2n = 2x = 14) and tetraploid cultivar 'Korok' of *L. perenne* and hexaploid cultivar 'Kora' (2n = 6x = 42) of *F. arundinacea*. Five plants of each species were used. With the aim of analyzing the distribution of homoeologous crossovers at the chromosomal level, single chromosome substitution lines of all 7 chromosomes of *F. pratensis* introgressed into the tetraploid *L. multiflorum* were developed [Kopecký et al., 2008]. Plants of individual chromosome substitutions were intercrossed and their progeny was analyzed.

Estimation of Nuclear Genome Size

Seeds were germinated in 12-cm pots filled with substrate in a greenhouse. After 3–4 weeks, we estimated the genome size of 5 plants of each cultivar by flow cytometry following Galbraith et al. [1983]. Nuclear suspensions were prepared by simultaneous chopping of 50 mg leaf tissue of an unknown sample and *Pisum sativum* 'Ctirad', which was used as an internal reference standard with 2C = 9.09 pg [Doležel et al., 1998]. The tissues were homogenized with a razor blade in a Petri dish containing 0.5 ml of Otto I solution (0.1 M citric acid, 0.5% Tween 20 [Otto, 1990]). The nuclear suspension was filtered through 42-μm nylon mesh and stained with 1 ml Otto II solution (0.4 M $Na_2HPO_412H_2O$) containing 2 mg/ml ß-mercaptoethanol, 50 μl/ml propidium iodide and 50 μl/ml RNase IIA. Samples were stained for 10 min at room temperature and analyzed using a CyFlow flow cytometer (Partec GmbH, Münster, Germany) equipped with a green solid-state laser (532 nm). At least 5,000 events were analyzed per sample and only histograms with a coefficient of variation of G_0/G_1 peaks lower than 4.0% were accepted. The analysis of each taxon was repeated on 3 different days. A between-day threshold of 2% was set and samples exhibiting larger variation were excluded and a new sample was analyzed.

Karyotyping of F. pratensis *and* L. multiflorum

To establish karyotypes of *F. pratensis* (cv. 'Kolumbus'; 2n = 2x = 14) and *L. multiflorum* (cv. 'Lubina'; 2n = 4x = 28), we measured chromosome lengths and determined centromeric indices in both species. Individual chromosomes were identified by fluorescence in situ hybridization (FISH) according to Kopecký et al. [2008] with labeled probes for 5S and 45S rDNA and the *F. pratensis* BAC clone 1G18. Chromosomes were numbered following the Triticeae Chromosome Numbering System [Kopecký et al., 2009b]. Chromosomes were counterstained with 1.5 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) prepared in Vectashield antifade solution (Vector Laboratories, Burlingame, Calif., USA). Observations were made using an Olympus AX70 microscope equipped with epifluorescence and SensiCam B/W camera. Chromosomes were measured in 10 metaphase plates in each species using ScionImage software, and mean values were calculated.

Distribution of Homoeologous Crossovers at the Chromosomal Level

Chromosome Preparations and Genomic in situ Hybridization. Single chromosome substitution lines *L. multiflorum*/*F. pratensis* were propagated by intermating within small populations of plants with the same chromosome constitutions, in isolation from all other sources of pollen. Seeds of the progeny thus obtained were germinated in Petri dishes on wet filter paper and seedlings were planted in 30-mm pots in the greenhouse. After 3–4 weeks, plantlets were transferred to a hydroponic culture with aerated solution of Hydroponex at 0.9 g/l (Hu-Ben, Čerčany, Czech Republic). Mitotic metaphase spreads were prepared from root tips according to Masoudi-Nejad et al. [2002]. GISH was performed according to Kopecký et al. [2005]. Total genomic DNA of *F. pratensis* was labeled with digoxigenin using the DIG-Nick Translation Kit (Roche Applied Science, Indianapolis, Ind., USA) and used as a probe. Genomic DNA of *L. multiflorum* was sheared to 200–500-bp fragments by boiling for 45 min and used as blocking DNA. The probe to block ratio was 1:150 with minor variation. Sites of probe hybridization were detected by anti-DIG-FITC con-

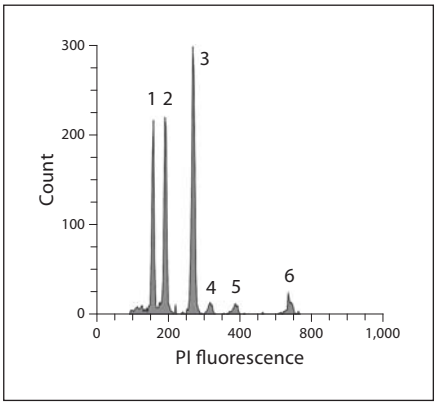


Fig. 1. Histogram of relative fluorescence intensity obtained after simultaneous analysis of nuclei isolated from *Lolium multiflorum* cultivar 'Prolog' (G_0/G_1 nuclei, peak 1; G_2 nuclei, peak 4), *Festuca pratensis* cultivar 'Kolumbus' (G_0/G_1 nuclei, peak 2; G_2 nuclei, peak 5) and *Pisum sativum* cultivar 'Ctirad' (2C = 9.09 pg, as an internal reference standard; G_0/G_1 nuclei, peak 3; G_2 nuclei, peak 6).

jugate (Roche). Fluorescence microscopy was performed as described above and ScionImage and Adobe Photoshop software were used for processing of color pictures.

Distribution of Homoeologous Recombination. To determine the positions of intergeneric crossover points, we measured the length of the introgressed segment(s) and the length of both arms of recombined chromosomes in 5 metaphase plates per plant. Because the absolute lengths of individual pairs of homologues from *Festuca* and *Lolium* are different, a direct calculation of the positions of crossover points along the chromosome length was not possible. Thus, we transformed the measured lengths of the introgressed *Festuca* segments using a calibration factor specific for each chromosome (table 1). The factor was calculated based on the DNA content and relative lengths of homoeologous chromosomes of both species:

Calibration factor = 1CxL × RLF/1CxF × RLL
1CxL = Monoploid genome size of *L. multiflorum*
1CxF = Monoploid genome size of *F. pratensis*
RLL = Relative length of individual *L. multiflorum* chromosome
RLF = Relative length of individual *F. pratensis* chromosome

For each chromosome, the position of over 100 independent homoeologous recombination events was recorded.

Results and Discussion

Estimation of Nuclear Genome Size

Nuclear DNA content of diploid and tetraploid cultivars of *L. multiflorum*, *L. perenne*, *F. pratensis* and of a hexaploid cultivar of *F. arundinacea* was estimated by flow cytometry (table 2). In agreement with the previous study [Loureiro et al., 2007], histograms of relative DNA content contained well-resolved peaks representing nuclei in G_0/G_1 and G_2 phases of the cell cycle (fig. 1). In 91.0% of the assays, the coefficient of variation (CV) of G_0/G_1 peaks was below 3.0% and average CV ranged from 1.96–2.96%. The analyses revealed that monoploid genome sizes (1Cx) [Greilhuber et al., 2005] of *F. pratensis* and *L. multiflorum* were significantly different. While both cultivars of *F. pratensis* measured here had 1Cx = 3.25 pg, diploid *L. multiflorum* cultivar 'Prolog' had 1Cx = 2.62 pg and the tetraploid cultivar 'Lubina' had 1Cx = 2.75 pg. The difference between the 2 *L. multiflorum* cultivars was statistically significant ($p < 0.05$). These observations suggest that, on average, the molecular size of an *F. pratensis* chromosome is 18% larger as compared to an *L. multiflorum* chromosome. This estimate was confirmed independently by the measurement of chromosome lengths (table 3). Estimation of genome size and chromosome lengths in both species was essential to obtain the calibration factor for the analyses of the physical distribution of homoeologous recombination.

With the aim of providing additional data on 1Cx values in *Festuca* and *Lolium* and average chromosome molecular size in the 2 genera, we estimated genome sizes in 1 cultivar of hexaploid *F. arundinacea* and in 2 cultivars of *L. perenne* of different ploidy. The 1Cx value for *F. arundinacea* was 11% lower as compared to that of *F. pratensis* (table 2). This difference could be due to the reduction in genome size known to take place following polyploidization [Leitch and Bennett, 2004], and which was also observed in *Festuca* [Seal, 1983; Loureiro et al., 2007]. On the other hand, the 1Cx values for both cultivars of *L. perenne* (table 2) were statistically not different from the estimates for *L. multiflorum* with the same ploidy level.

Table 1. Calibration factor for the measurements of individual homoeologous chromosomes of *F. pratensis* and *L. multiflorum*

Chromosome	Calibration factor[a]
1	0.792
2	0.863
3	0.877
4	0.862
5	0.801
6	0.767
7	0.914

[a] Molecular size of *L. multiflorum* chromosome/molecular size of *F. pratensis* chromosome. For calculation, see Material and Methods.

Table 2. Estimation of nuclear genome size in *Lolium perenne*, *L. multiflorum*, *Festuca arundinacea* and *F. pratensis*

Species	Cultivar	2C Nuclear DNA amount, pg		1Cx, pg DNA	1Cx, Mb
		mean	SD (n = 5)		
L. multiflorum (2n = 2x = 14)	Prolog	5.25	0.042	2.62[a]	2,567
L. perenne (2n = 2x = 14)	Handicap	5.36	0.047	2.68[a, b]	2,623
L. multiflorum (2n = 4x = 28)	Lubina	10.99	0.092	2.75[b, c]	2,687
L. perenne (2n = 4x = 28)	Korok	11.19	0.057	2.80[c]	2,735
F. arundinacea (2n = 6x = 42)	Kora	17.45	0.078	2.91[d]	2,845
F. pratensis (2n = 2x = 14)	Kolumbus	6.49	0.078	3.25[e]	3,175
F. pratensis (2n = 4x = 28)	Patra	13.01	0.050	3.25[e]	3,181

1Cx = Monoploid genome size. 1 pg DNA = 978 Mb [Doležel et al., 2003].

Small letters label groups with no significant difference at $p < 0.05$ level according to a Bonferroni all pairwise multiple comparisons test.

Table 3. Length, DNA content and molecular size of chromosomes and chromosome arms of *F. pratensis* (cv. 'Kolumbus', 2n = 2x = 14) and *L. multiflorum* (cv. 'Lubina', 2n = 4x = 28)

Chr.	Length μm	% of total genome	1C pg	1C Mb[a]	Chr. arm	Length μm	% of total genome	1C pg	1C Mb[a]	Chr. arm	Length μm	% of total genome	1C pg	1C Mb[a]	CI[b]
F. pratensis Huds.															
1	4.67	11.74	0.382	373	**1S**	1.71	4.30	0.140	137	**1L**	2.96	7.44	0.242	237	37
2	6.07	15.24	0.496	485	**2S**	2.42	6.09	0.198	194	**2L**	3.64	9.15	0.298	291	40
3[c]	6.25	15.70	0.511	499	**3S**	2.92	7.34	0.239	233	**3L**	3.33	8.36	0.272	266	47
4	6.79	17.07	0.555	543	**4S**	3.18	7.99	0.260	254	**4L**	3.61	9.08	0.295	289	47
5	5.04	12.68	0.412	403	**5S**	1.76	4.41	0.143	140	**5L**	3.29	8.27	0.269	263	35
6	4.93	12.38	0.403	394	**6S**	1.97	4.95	0.161	158	**6L**	2.95	7.42	0.241	236	40
7	6.05	15.21	0.495	484	**7S**	2.90	7.28	0.237	231	**7L**	3.16	7.93	0.258	252	48
L. multiflorum Lam.															
1	3.37	11.01	0.303	296	**1S**	1.28	4.18	0.115	112	**1L**	2.09	6.83	0.188	183	38
2[c]	4.77	15.58	0.428	419	**2S**	1.66	5.42	0.149	146	**2L**	3.11	10.16	0.279	273	35
3[c]	4.99	16.30	0.448	438	**3S**	2.37	7.74	0.213	208	**3L**	2.62	8.56	0.235	230	47
4	5.33	17.41	0.479	468	**4S**	2.54	8.30	0.228	223	**4L**	2.79	9.11	0.251	245	48
5	3.68	12.02	0.331	323	**5S**	1.20	3.92	0.108	105	**5L**	2.48	8.10	0.223	218	33
6	3.44	11.23	0.309	302	**6S**	1.47	4.80	0.132	129	**6L**	1.97	6.43	0.177	173	43
7[c]	5.04	16.46	0.453	442	**7S**	2.31	7.54	0.207	203	**7L**	2.73	8.92	0.245	240	46

[a] 1 pg DNA = 978 Mb [Doležel et al., 2003].
[b] CI = Centromeric index (length of the short arm/total chromosome length × 100).
[c] Chromosomes with NOR.

This suggests that the average chromosome molecular size in *L. multiflorum* and *L. perenne* are similar.

The Karyotypes of F. pratensis *and* L. multiflorum

Measurements of chromosome length on 10 metaphase plates in each species established standard karyotypes for the 2 parental species (table 3). Diploid *F. pratensis* has 7 pairs of chromosomes of which 3 are metacentric and 4 are submetacentric; of the metacentric chromosomes, 1 pair (chromosome 3) has a secondary constriction. The length of the chromosomes ranges from 4.67 μm (chromosome 1) to 6.79 μm (chromosome 4) and the total length of the chromosome complement was 39.79 μm. Based on the estimated 1Cx value in *F. pra-*

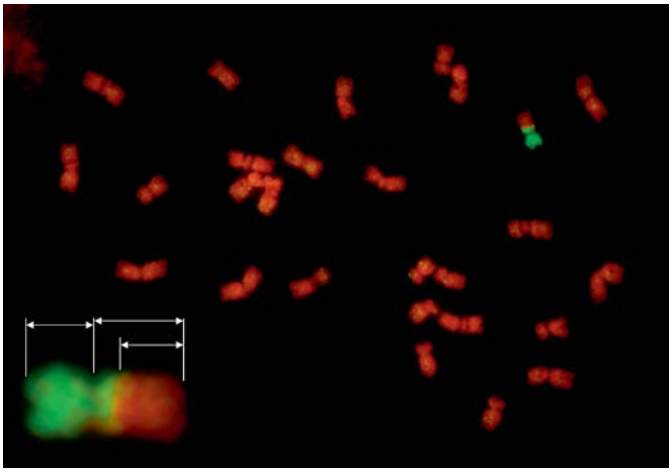


Fig. 2. Molecular cytogenetics analysis (GISH) of chromosomes in recombinant line Bdel21 (2n = 4x = 27), where a segment of *F. pratensis* chromosome 2 has been introgressed into tetraploid *L. multiflorum*. Total genomic DNA of *F. pratensis* was labeled with FITC and used as a probe (green color); unlabeled total genomic DNA of *L. multiflorum* was used as blocking DNA. Chromosomes were counterstained with DAPI (shown in red pseudocolor). Inset shows the chromosome measurement in detail.

tensis, the molecular size of individual chromosomes ranges from approximately 373–543 Mb. Diploid *L. multiflorum* also has 7 pairs of chromosomes; 3 pairs are metacentric and 4 pairs are submetacentric. Three pairs of chromosomes (chromosomes 2, 3 and 7) carry 1 secondary constriction each. The length of individual chromosomes ranges from 3.37 μm in chromosome 1 to 5.33 μm in chromosome 4, and molecular sizes of chromosomes range from 296 Mb (chromosome 1) to 468 Mb (chromosome 4). The length of the entire chromosome complement was 30.62 μm.

Distribution of Homoeologous Recombination Points

To establish the distribution of recombination points along individual chromosomes, we divided each chromosome into 20 segments of equal size. Each segment corresponded to approximately 20 Mb of DNA and its size was chosen considering the spatial resolution of our microscopy-camera system, where 1 pixel represents 0.98–1.43% of a chromosome length (fig. 2). Measurements of recombined chromosomes in 5 different metaphase plates showed that variation in length of up to 5% was not uncommon (data not shown). This variation reflected differences in chromatin condensation among different cells, differences in the labeling efficiency of FISH, and spatial resolution of our optical detection system. Interestingly, King et al. [2002] were able to dissect a chromosome into segments smaller than 0.4% of its entire physical length.

The frequency of recombination events was established for each of the 20 segments in each single chromosome substitution line. The results are graphically displayed in figure 3 in which the chromosome lengths are given in relative values, so that the positions of recombination events are valid for both species. In most of the chromosome arms, the distribution of recombination events was fairly even with a slow gradient of increasing recombination rates towards the telomeres. However, there was a dramatic decrease in recombination at the subtelomeric and telomeric regions. In each chromosome, the lowest recombination rates were in the pericentromeric and centromeric regions. Absence of recombination in the centromeric/pericentromeric and telomeric/subtelomeric regions was not entirely surprising as it has been reported in triploid hybrids *L. multiflorum* × *F. pratensis* and in the pentaploid hybrids *F. arundinacea* × *L. multiflorum* [Zwierzykowski et al., 1998, 1999].

Gradients of recombination along chromosomes were found in other grass species, including wheat [Dvořák and Chen, 1984; Curtis and Lukaszewski, 1991; Erayman et al., 2004], rye [Lukaszewski, 1992], barley [Leitch and Heslop-Harrison, 1993; Künzel et al., 2000] and ryegrass [Hayward et al., 1998]. In barley, Künzel et al. [2000] found that 45% of the physical length of chromosome 3H spanning the centromere showed no recombination at all, while in wheat, Erayman et al. [2004] observed less than 1% of overall chromosome recombination in the 25% around the centromere. Some of the decrease in recombination rates in the vicinity of the telomere may be attributed to detection problems. Given the resolution level of FISH [Lukaszewski et al., 2005] it is possible that very short segments of *F. pratensis* chromatin on *L. multiflorum* chromosomes did not produce bright enough signals to be observed while short terminal segments of *L. multiflorum* on *F. pratensis* chromosomes might have been obscured by the halo of the probe. There are no conceivable technical problems in scoring all remaining intercalary crossover events, including those in the vicinity of the centromere.

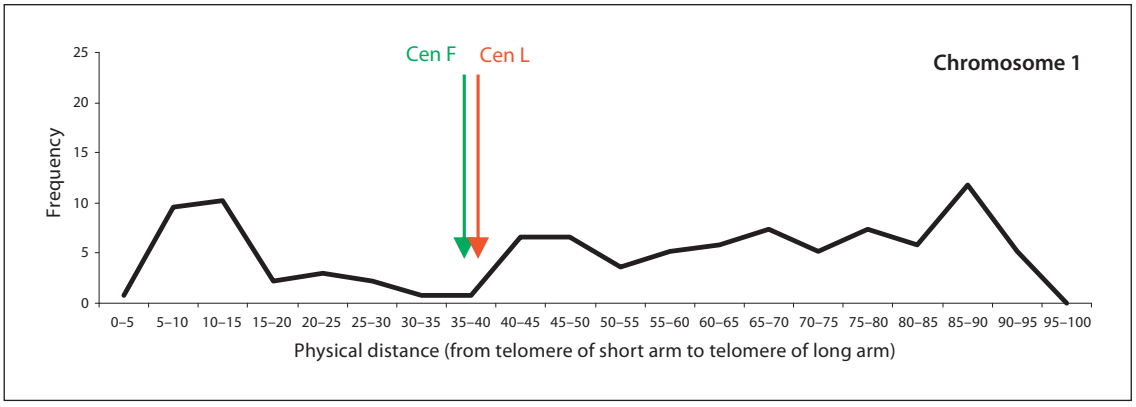
A reduced frequency of recombination was observed to correlate with the presence of the nucleolus organizing regions (NOR) and was observed in NOR-bearing arms such as wheat chromosome 1BS [Dvořák and Chen, 1984], rye chromosome 1RS [Lawrence and Appels, 1986] and chromosome 3 of the *L. perenne*/*F. pratensis* substitution line [King et al., 2002]. The present study indicates that

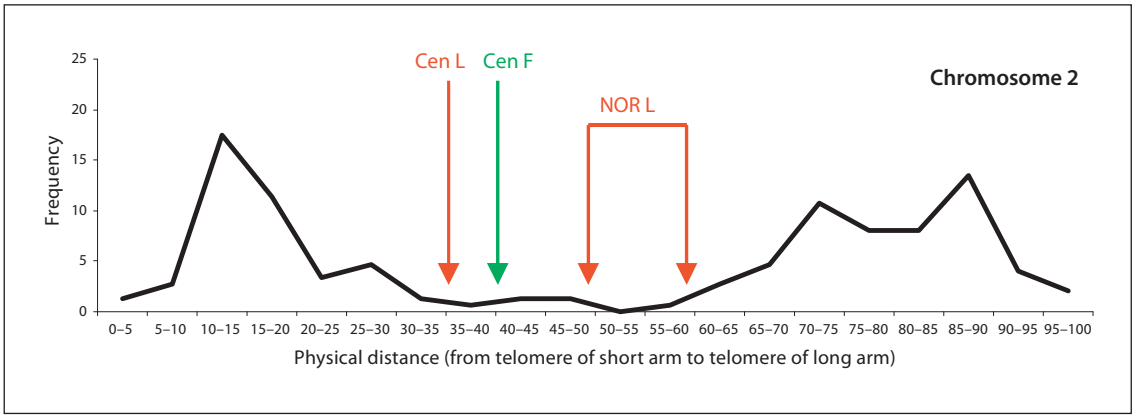
the relationship between the presence of a NOR and recombination may be more complex. While we did not observe any reduction of homoeologous recombination in the NOR region of chromosome 3, chromosome 7 had lower frequency of recombination in the NOR region, and chromosome 2 showed an absence of recombination in the vicinity of the NOR. In the case of chromosome 3 of *F. pratensis*, the differences observed between our results and those of King et al. [2002] might have resulted from the lower sequence homology between *F. pratensis* and *L. perenne* [King et al., 2002] as compared to *F. pratensis* and *L. multiflorum* (this study). In fact, our previous study indicated a closer relationship between the latter 2 species [Kopecký et al., 2009b]. The sequence homology could also explain the differences in recombination observed in chromosome 3 and chromosomes 2 and 7. While chromosome 3 of both parental species *(L. multiflorum* and *F. pratensis)* bears NORs, chromosomes 2 and 7 have NORs only in *L. multiflorum*. Thus, the absence of recombination could be due to structural differences rather than the presence of the NOR per se. This issue clearly deserves further study.

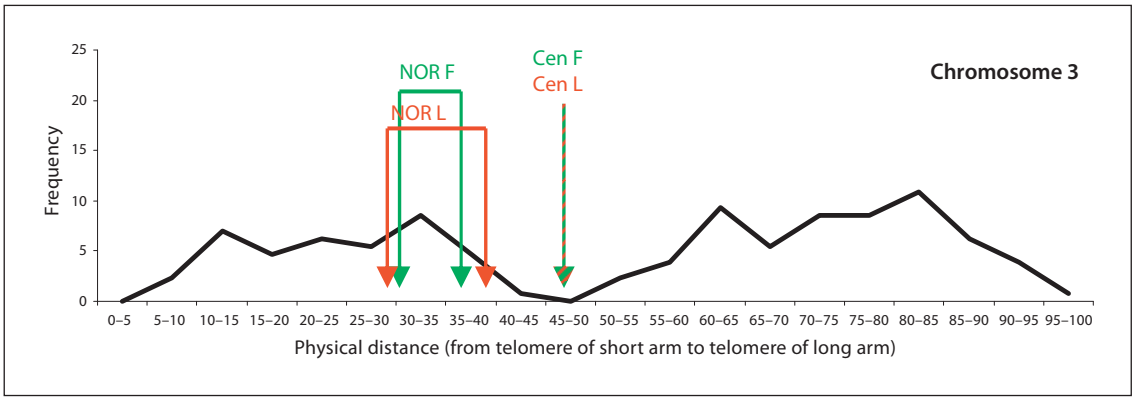
Both *F. pratensis* and *L. multiflorum* are species with typical distal chiasmata producing distinct ring bivalents and end-to-end paired rods [Rees and Dale, 1974; Karp and Jones, 1983]. Thus, the low frequency of homoeologous recombination in the telomeric/subtelomeric regions of *Festuca* × *Lolium* hybrids is difficult to explain. Some part of it could be related to detection problems, as explained earlier. However, the observations of Zwierzykowski et al. [1998, 1999] and King et al. [2002] confirm that this phenomenon is typical for the *Festuca* × *Lolium* hybrids. The decrease in frequency of recombination was also observed at the very distal end of the short arm of wheat chromosome 3B [Saintenac et al., 2009]. As in other chromosomal regions mentioned above, the explanation could be due to lower sequence homology. In hexaploid wheat, Akhunov et al. [2003b] observed a gradient in gene density and recombination rate from the centromere to the telomere of all chromosomes. Higher frequency of recombination may explain a more rapid gene evolution and faster differentiation of homoeologous chromosomes from each other in distal regions [Akhunov et al., 2003a]. As homoeologous chromosomes of cross-pollinating species lose synteny faster than do homoeologues of self-pollinating species [Akhunov et al., 2003a], it is reasonable to expect reduced sequence homology between the distal parts of homoeologous chromosomes of *F. pratensis* and *L. multiflorum*. Moreover, the presence of telomere-associated repeats, which are presumably under low selection pressure, provides another source of sequence variation. Telomeric heterochromatin was suggested as a possible reason for the decrease of recombination in the distal part of the short arm of wheat chromosome 3B [Saintenac et al., 2009]. On the other hand, if the hypothesis of zygomeres or pairing centers is ever confirmed [Sybenga, 1969], many aspects of the particular crossover patterns would be easier to understand and explain.

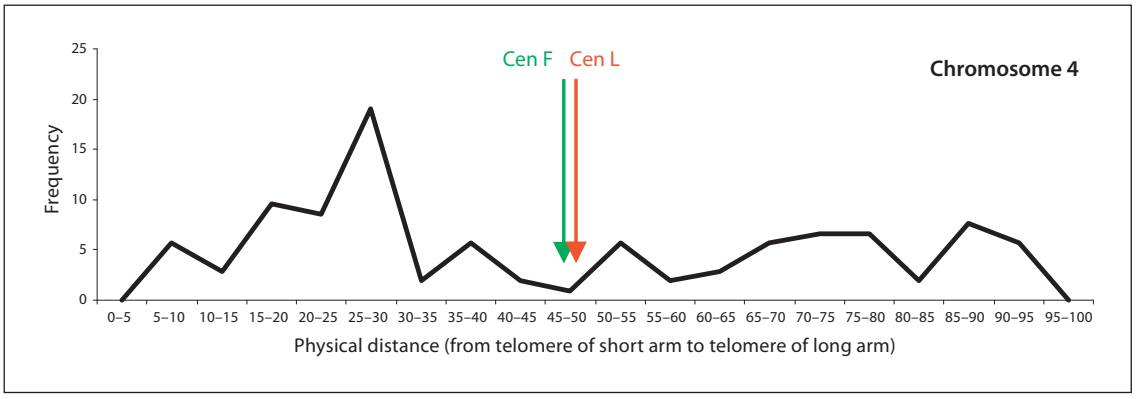
Uneven distribution of recombination along chromosomes was described in a number of studies [Mézard, 2006; Buard and de Massy, 2007]. The molecular nature of the regions with an unusually high or low frequency of recombination is unclear [Akhunov et al., 2003; Mézard, 2006]. In this work, recombination hotspots and cold spots were not frequently observed. Hotspots occurred in the central parts of chromosome arms and were usually located closer to telomeres. Hotspots with a frequency of recombination 4 times higher than the average occur at a physical distance of 25–30% in chromosome 4 and 85–90% in chromosome 6 (fig. 3). Cold spots, or absence of recombination, were generally localized to regions neighboring centromeres. Moreover, several other parts of chromosomes displayed reduced frequency of recombination. Unexpected cold spots occurred for example at a physical distance of 75–80% of chromosome 5 and between 30 and 35% of chromosome 6 (fig. 3). The cumulative length of regions with no recombination, excluding the most distal segments, ranged from 0% in chromosomes 1, 4 and 5 to 15% in chromosomes 6 and 7. The reduction of recombination frequency in the regions around the NOR in chromosomes 2 and 7 was already mentioned.

This study provides perhaps the most detailed map of homoeologous recombination in the *Festuca-Lolium* complex. In general, recombination frequency is the highest in the distal chromosome regions and decreases toward the centromere. In this sense, the pattern is similar to that observed in numerous other species. How valid this pattern is for homologous recombination is not clear. While the *Festuca-Lolium* complex offers a unique opportunity to visualize the parental genomes, chromosomes, and small chromosome segments in large numbers of progenies produced in a variety of ways, no such system exists for homologous recombination. The relationship between homologous and homoeologous recombination has never been established in any convincing form. In wheat, homoeologous recombination follows the same exact pattern of frequency distribution from the telomere toward the centromere, with the exception of


Chromosome 1
Cen F
Cen L
Frequency
0
5
10
15
20
25
0–5 5–10 10–15 15–20 20–25 25–30 30–35 35–40 40–45 45–50 50–55 55–60 60–65 65–70 70–75 75–80 80–85 85–90 90–95 95–100
Physical distance (from telomere of short arm to telomere of long arm)


Chromosome 2
Cen L
Cen F
NOR L
Frequency
0
5
10
15
20
25
0–5 5–10 10–15 15–20 20–25 25–30 30–35 35–40 40–45 45–50 50–55 55–60 60–65 65–70 70–75 75–80 80–85 85–90 90–95 95–100
Physical distance (from telomere of short arm to telomere of long arm)


Chromosome 3
NOR F
NOR L
Cen F
Cen L
Frequency
0
5
10
15
20
25
0–5 5–10 10–15 15–20 20–25 25–30 30–35 35–40 40–45 45–50 50–55 55–60 60–65 65–70 70–75 75–80 80–85 85–90 90–95 95–100
Physical distance (from telomere of short arm to telomere of long arm)


Chromosome 4
Cen F
Cen L
Frequency
0
5
10
15
20
25
0–5 5–10 10–15 15–20 20–25 25–30 30–35 35–40 40–45 45–50 50–55 55–60 60–65 65–70 70–75 75–80 80–85 85–90 90–95 95–100
Physical distance (from telomere of short arm to telomere of long arm)

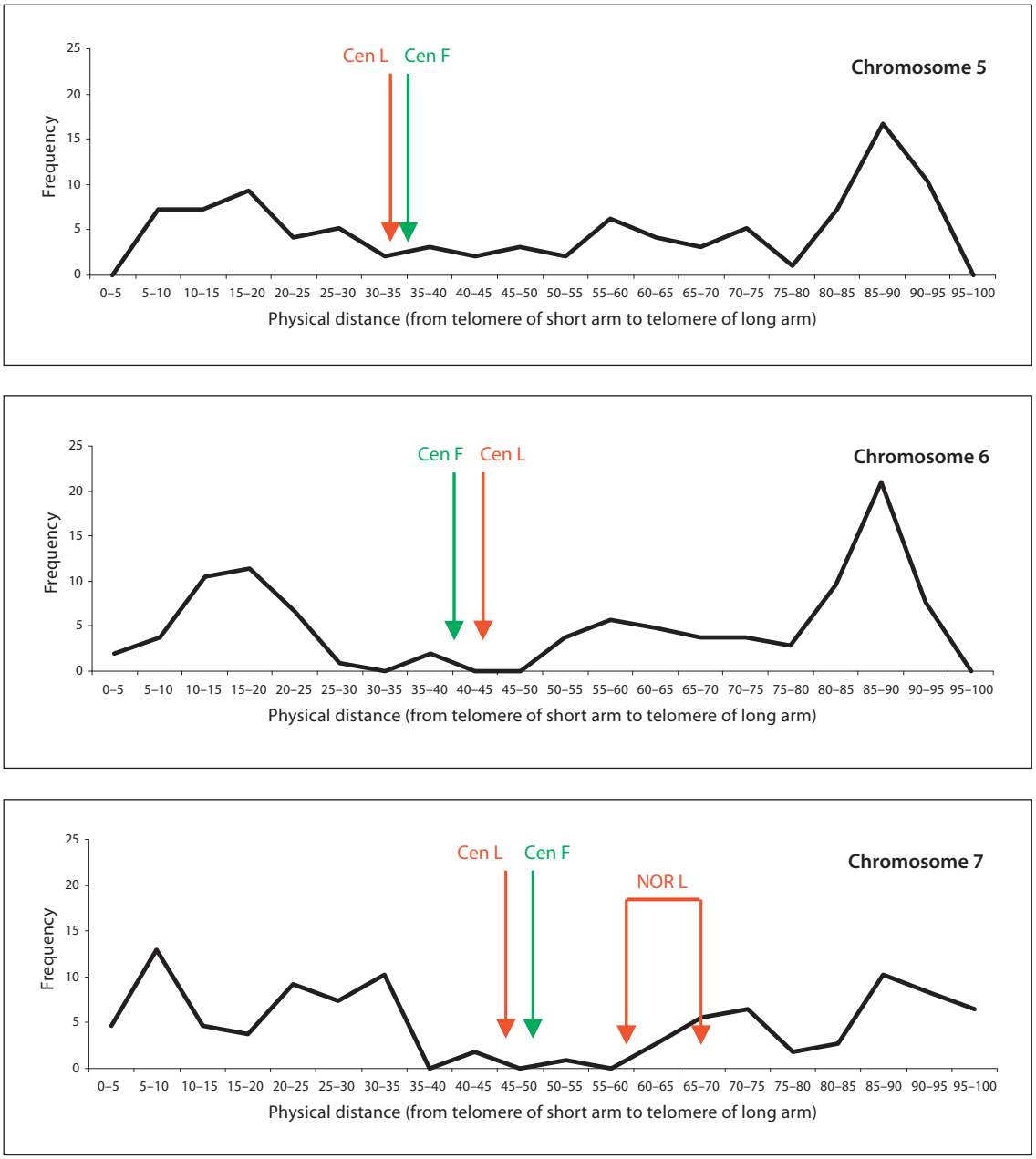


Fig. 3. Distribution of homoeologous recombination events along individual chromosomes of *L. multiflorum*/*F. pratensis* substitutions. Each chromosome is divided into 20 segments, each representing 5% of the physical length. The position of centromeres and NORs in parental species is marked by red *(L. multiflorum)* and green arrows *(F. pratensis)*.

the virtual absence of multiple crossovers per arm [Lukaszewski, 1995], but the pattern does not necessarily indicate much similarity at the DNA level. Moreover, homoeologous recombination in wheat is infrequent, even in the absence of the *Ph1* locus. In the grasses studied here, homoeologues pair and recombine so frequently that pairing preferences require considerable samples to be detected [Kopecký et al., 2008]. From this perspective, chances that similar chromosome regions are involved in homologues as in homoeologues seem higher. But then again, the human-chimpanzee example of recombination hotspots demonstrates that their relative importance

may change over time and so in the species studied here they may not be equivalent or even the same.

While this study detected some regions of higher and lower recombination, crossover events occurred along the entire lengths of chromosomes. Thus, in principle, introgression of any chromatin segment from the donor parental species *(Festuca)* into the recipient one *(Lolium)* should be possible, but not necessarily equally simple. However, introgression breeding can attempt to handle any trait of interest considering that the final success will depend on the ability to control the size of introgressed segments to avoid linkage drag.

Acknowledgement

This work was supported by the Ministry of Agriculture of the Czech Republic (grant award NAZV QH71267) and by the Czech Science Foundation (grant award 521/07/P479).

.

References

Akhunov ED, Akhunova AR, Linkiewicz AM, Dubcovsky J, Hummel D, et al: Synteny perturbations between wheat homoeologous chromosomes caused by locus duplications and deletions correlate with recombination rates. Proc Natl Acad Sci USA 100:10836–10841 (2003a).

Akhunov ED, Goodyear AW, Geng S, Qi LL, Echalier B, et al: The organization and rate of evolution of wheat genomes are correlated with recombination rates along chromosome arms. Genome Res 13:753–763 (2003b).

Anderson LK, Lai A, Stack SM, Rizzon C, Gaut BS: Uneven distribution of expressed sequence tag loci on maize pachytene chromosomes. Genome Res 16:115–122 (2006).

Buard J, de Massy B: Playing hide and seek with mammalian meiotic crossover hotspots. Trends Genet 23:301–309 (2007).

Catalán P, Torrecilla P, Rodriguez JAL, Olmstead RG: Phylogeny of the festucoid grasses of subtribe *Loliinae* and allies (Poeae, Pooideae) inferred from ITS and trnL-F sequences. Mol Phylogenet Evol 31:517–541 (2004).

Černoch V, Našinec I, Šramek P: Share of grassland on landscape forming in the Czech Republic. Czech J Genet Plant Breed 39:158–162 (2003).

Curtis CA, Lukaszewski AJ: Genetic linkage between C-bands and storage protein genes of tetraploid wheat. Theor Appl Genet 81:245–252 (1991).

Doležel J, Greilhuber J, Lucretti S, Meister A, Lysak M, et al: Plant genome size estimation by flow cytometry: inter-laboratory comparison. Ann Bot 82:17–26 (1998).

Dooner HK, Martinez-Ferez IM: Recombination occurs uniformly within the bronze gene, a meiotic recombination hotspot in the maize genome. Plant Cell 9:1933–1646 (1997).

Drouaud J, Camilleri C, Bourguignon PY, Canaguier A, Bérard A, et al: Variation in crossing-over rates across chromosome 4 of *Arabidopsis thaliana* reveals the presence of meiotic recombination 'hotspots'. Genome Res 16:106–114 (2006).

Dvořák J, Chen KY: Distribution of nonstructural variation between wheat cultivars along chromosome 6Bp: evidence from the linkage map and physical map of the arm. Genetics 106:325–333 (1984).

Erayman MD, Sandhu D, Sidhu H, Dilbirligi M, Baenziger PS, Gill KS: Demarcating the gene-rich regions of the wheat genome. Nucleic Acids Res 32:3546–3565 (2004).

Fojtík A: Methods of grass improvement used at the plant breeding station Hladké Životice. Genet Pol 35A:25–31 (1994).

Francis KE, Lam SY, Harrison BD, Bey AL, Berchowitz LE, Copenhaver GP: Pollen tetrad-based visual assay for meiotic recombination in *Arabidopsis*. Proc Natl Acad Sci USA 104: 3913–3918 (2007).

Galbraith DW, Harkins KR, Maddox JM, Ayres NM, Sharma DP, Firoozabady E: Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science 220:1049–1051 (1983).

Gill KS, Gill BS, Endo TR, Taylor T: Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. Genetics 144:1883–1891 (1996).

Greilhuber J, Doležel J, Lysák MA, Bennett MD: The origin, evolution and proposed stabilization of the terms 'genome size', and 'C-value' to describe nuclear DNA contents. Ann Bot 95:255–260 (2005).

Hayward MD, Forster JW, Jones JG, Dolstra O, Evans C, et al: Genetic analysis of *Lolium* I. Identification of linkage groups and the establishment of a genetic map. Plant Breed 117:451–455 (1998).

Jauhar PP: Cytogenetics of the *Festuca-Lolium* complex. Relevance to Breeding. Monographs on Theor Appl Genet Vol. 18, p 255 (Springer-Verlag, Berlin 1993).

Jeffreys AJ, Neumann R: Factors influencing recombination frequency and distribution in a human meiotic crossover hotspot. Hum Mol Genet 37:601–606 (2005).

Jenczewski E, Eber F, Grimaud A, Huet S, Lucas MO, et al: *PrBn*, a major gene controlling homeologous pairing in oilseed rape *(Brassica napus)* haploids. Genetics 164:645–653 (2003).

Jones ES, Dupal MP, Dumsday JL, Hughes LJ, Forster JW: An SSR-based genetic linkage map for perennial ryegrass (*Lolium perenne* L.). Theor Appl Genet 105:577–584 (2002).

Jones LE, Rybka K, Lukaszewski AJ: The effect of a deficiency and a deletion on recombination in chromosome 1BL in wheat. Theor Appl Genet 104:1204–1208 (2002).

Karp A, Jones RN: Cytogenetics of *Lolium perenne*. Part 2. Chiasma distribution in inbred lines. Theor Appl Genet 64:137–145 (1983).

King J, Armstead IP, Donnison IS, Thomas HM, Jones RM, et al: Physical and genetic mapping in the grasses *Lolium perenne* and *Festuca pratensis*. Genetics 161:315–324 (2002).

King J, Armstead IP, Donnison IS, Harper JA, Roberts LA, et al: Introgression mapping in the grasses. Chromosome Res 15:105–113 (2007).

Kopecký D, Lukaszewski AJ, Doležel J: Genomic constitution of Festulolium cultivars released in the Czech Republic. Plant Breed 124:454–458 (2005).

Kopecký D, Lukaszewski AJ, Doležel J: Meiotic behavior of individual chromosomes of *Festuca pratensis* in tetraploid *Lolium multiflorum*. Chromosome Res 16:987–998 (2008).

Kopecký D, Bartoš J, Zwierzykowski Z, Doležel J: Chromosome pairing of individual genomes in tall fescue (*Festuca arundinacea* Schreb.), its progenitors, and hybrids with Italian ryegrass (*Lolium multiflorum* Lam.). Cytogenet Genome Res 124:170–178 (2009a).

Kopecký D, Bartoš J, Lukaszewski AJ, Baird JH, Cernoch V, et al: Development and mapping of DArT markers within the *Festuca - Lolium* complex. BMC Genomics 10:473 (2009b).

Künzel G, Korzum L, Meister A: Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics 154:397–412 (2000).

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N: A 300-kilobase interval genetic map of rice including 883 expressed sequences. Nat Genet 8:365–372 (1994).

Lawrence GJ, Appels R: Mapping the nucleolar organiser region, seed protein loci and isozyme loci on chromosome 1R in rye. Theor Appl Genet 71:742–749 (1986).
Leitch IJ, Bennett MD: Genome downsizing in polyploid plants. Biol J Linn Soc 82:651–663 (2004).
Leitch IJ, Heslop-Harrison JS: Physical mapping of 4 sites of 5S rRNA sequences and one site of the *α-amylase-2* gene in barley *(Hordeum vulgare)*. Genome 36:517–523 (1993).
Lenormand T, Dutheil J: Recombination difference between sexes: a role for haploid selection. PLoS Biol 3:e63 (2005).
Loureiro J, Kopecký D, Castro S, Santos C, Silveira P: Flow cytometric and cytogenetic analyses of Iberian Peninsula *Festuca* spp. Plant Syst Evol 269:89–105 (2007).
Lukaszewski AJ: A comparison of physical distribution of recombination in chromosome 1R in diploid rye and hexaploid triticale. Theor Appl Genet 83:1048–1053 (1992).
Lukaszewski AJ: Physical distribution of translocation breakpoints in homoeologous recombinants induced by the absence of the *Ph1* gene in wheat and triticale. Theor Appl Genet 90:714–719 (1995).
Lukaszewski AJ, Curtis CA: Physical distribution of recombination in B-genome chromosomes of tetraploid wheat. Theor Appl Genet 86:121–127 (1993).
Lukaszewski AJ, Lapinski B, Rybka K: Limitations of in situ hybridization with total genomic DNA in routine screening for alien introgressions in wheat. Cytogenet Genome Res 109:373–377 (2005).
Maguire MP: The pattern of pairing that is effective for crossing over in complex B-A chromosome rearrangements in maize. III. Possible evidence for pairing centers. Chromosoma 94:71–85 (1986).
Masoudi-Nejad A, Nasuda S, McIntosh RA, Endo TR: Transfer of rye chromosome segments to wheat by a gametocidal system. Chromosome Res 10:349–357 (2002).
Mézard C: Meiotic recombination hotspots in plants. Biochem Soc Trans 34:531–534 (2006).
Okagaki RJ, Weil CF: Analysis of recombination sites within the maize waxy locus. Genetics 147:815–821 (1997).
Otto F: DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA, in Crissman HA, Darzynkiewicz Z (eds): Methods in Cell Biology, vol. 33, pp 105–110 (Academic Press, New York 1990).
Qi LL, Friebe B, Gill BS: A strategy for enhancing recombination in proximal regions of chromosomes. Chromosome Res 10:645–654 (2002).
Rees H, Dale PJ: Chiasmata variability in *Lolium* and *Festuca* populations. Chromosoma 47: 335–351 (1974).
Riley R, Chapman V: Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature 182:713–715 (1958).
Saintenac C, Falque M, Martin OC, Paux E, Feuillet C, Sourdille P: Detailed recombination studies along chromosome 3B provide new insights on crossover distribution in wheat (*Triticum aestivum* L.). Genetics 181: 393–403 (2009).
Sall T, Flink J, Bengtsson BO: Genetic-control of recombination in barley. 1. Variation in recombination frequency measured with inversed heterozygotes. Hereditas 112:157–170 (1990).
Seal AG: DNA variation in *Festuca*. Heredity 50: 225–236 (1983).
Sears ER, Okamoto M: Intergenomic chromosome relationships in hexaploid wheat. Proc 10th Int Congr Genet, Montreal 2:258–259 (1958).
Shah MM, Hassan A: Distribution of genes and recombination on wheat homoeologous group 6 chromosomes: a synthesis of available information. Mol Breed 15:45–53 (2005).
Sinha RP, Helgason SB: Action of actinomycin D and diepoxybutane on recombination of 2 closely linked loci in *Hordeum*. Canad J Genet Cytol 11:745 (1969).
Sybenga J: The zygomere as hypothetical unit of chromosome pairing initiation. Genetica 37: 186–198 (1969).
Sybenga J: Meiotic Configurations (Springer-Verlag, Berlin 1975).
Thomas HM, Morgan WG, Meredith MR, Humphreys MW, Thomas H, Leggett JM: Identification of parental and recombined chromosomes in hybrid derivatives of *Lolium multiflorum* × *Festuca pratensis* by genomic in situ hybridization. Theor Appl Genet 88: 909–913 (1994).
Winckler W, Myers SR, Richter DJ: Comparison of fine-scale recombination rates in humans and chimpanzees. Science 308:107–111 (2005).
Zwierzykowski Z, Lukaszewski AJ, Lesniewska A, Naganowska B: Genomic structure of androgenic progeny of pentaploid hybrids, *Festuca arundinacea* × *Lolium multiflorum*. Plant Breed 117:457–462 (1998).
Zwierzykowski Z, Lukaszewski AJ, Naganowska B, Lesniewska A: The pattern of homoeologous recombination in triploid hybrids of *Lolium multiflorum* with *Festuca pratensis*. Genome 42:720–726 (1999).