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Chromosome pairing of individual genomes in tall fescue (*Festuca arundinacea* Schreb.), its progenitors, and hybrids with Italian ryegrass (*Lolium multiflorum* Lam.)

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Abstract

A diploid-like pairing system prevents meiotic irregularities and improves the efficiency of gamete production in allopolyploid species. While the nature of the system is known in some polyploid crops including wheat, little is known about the control of chromosome pairing in polyploid fescues (*Festuca* spp.). In this work we studied chromosome pairing in allohexaploid *F. arundinacea,* its progenitors *F. pratensis* and *F. glaucescens,* and two intergeneric hybrids *Lolium multiflorum* (2x) × *F. arundinacea* (6x) and *L. multiflorum* (4x) × *F. glaucescens* (4x). The use of genomic in situ hybridization (GISH) permitted the analysis of homoeologous chromosome pairing and recombination of different genomes involved. We detected a diploid-like pairing system in polyploid fescues *F. arundinacea* and *F. glaucescens,* the latter being one of the progenitors of *F. arundinacea.* The pairing control system was absent in the second progenitor *F. pratensis.* Detailed analysis of intergeneric hybrids confirmed the presumed haploinsufficiency of the fescue system, which resulted in homoeologous pairing between all component genomes. This indicates that introgression of any specific chromosome segment from one genome to another is possible in all genome combinations. Our results not only contribute to the quest to discover the nature of the system controlling chromosome pairing in polyploid fescues, but may also have serious implications for design of hybrid breeding schemes in forage grasses.

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Tall fescue (*Festuca arundinacea* Schreb.) is a perennial grass species with a wide distribution over Europe, North-West Africa, and temperate areas of Asia. It has also been introduced into North America and is now grown commercially on a considerable acreage (Cernoch et al., 2003). Tall fescue is widely grown for forage, both as a monoculture and in mixture with other grasses. Its turf use has increased dramatically in recent decades. *F. arundinacea* is known for its ability to survive summer drought, and, relative to other grasses, it is well adapted to low winter temperatures. Its disadvantages relative to ryegrasses (*Lolium multiflorum* Lam. and *L. perenne* L.) are the slow establishment from seed, low tillering density, low palatability, and low biomass production in the first year (Jauhar, 1993).

To mitigate the deficiencies of *F. arundinacea,* grass breeders resort to wide hybridization, with the partners of choice being *L. multiflorum* (Italian ryegrass) and *L. perenne* (perennial ryegrass). The two ryegrass species belong to the most important forage and turf grasses. In contrast to tall fescue they have good digestibility and palatability and rapidly establish from seed. Cultivars of *L. perenne* used in turf are characterized by dark green color, good density, texture, and uniformity. On the oth-

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er hand, both ryegrass species are sensitive to abiotic stresses. Therefore, wide hybridization of *Festuca* and *Lolium* species offers a chance to complement the agronomic profiles of both parents.

Fescues and ryegrasses hybridize in nature, but their hybrids are nearly completely sterile. In breeding programs, the F1 hybrid sterility can be overcome by chromosome doubling, and fertile hybrid cultivars have been produced. One of the first hybrid cultivars combining superior characteristics of *Lolium* and *Festuca* was cv. 'Kenhy', developed by Prof. R.C. Buckner and his group (Buckner et al., 1977). The breeding scheme involved production of allooctoploids *L. multiflorum* (2x) × *F. arundinacea* (6x), which were backcrossed to *F. arundinacea*. The cultivar 'Kenhy' became a commercial success and was used for a long time either in pure stands or in mixtures with *F. arundinacea* cultivars. A similar breeding strategy was employed by Fojt'k (1994) who crossed *L. multiflorum* with *F. arundinacea* and developed hybrid forage cultivars 'Lofa', 'Bečva' (after backcrossing to tetraploid *L. multiflorum*), 'Hykor', 'Felina', 'Fojtan' and turf cultivars 'Korina' and 'Lesana' (after backcrossing to *F. arundinacea*).

F. arundinacea is allohexaploid (2n = 6x = 42) and it apparently evolved by spontaneous hybridization of *F. pratensis* Huds. (2n = 2x = 14) with tetraploid *F. arundinacea* var. *glaucescens* Boiss. (2n = 4x = 28) (referred here to as *F. glaucescens*). *F. glaucescens* in turn is apparently composed of two slightly diverged genomes Fg and Fg′ (Humphreys et al., 1995; Thomas et al., 1997), and the genomic constitution of *F. arundinacea* is thus FpFpFgFgFg′Fg′. The component genomes are closely related and their chromosomes pair in metaphase I (MI) of meiosis with a high frequency in most intergeneric hybrids within the *Lolium-Festuca* complex (Jauhar, 1975a). Nevertheless, cytologically *F. arundinacea* itself behaves like a diploid, forming only bivalents in MI (Lewis et al., 1980), suggesting the presence of a diploidizing genetic system similar to other allopolyploids such as wheat (Riley and Chapman, 1958), oat (Gauthier and McGinnis, 1968), and polyploid *Agropyron* species (Charpentier et al., 1986). Jauhar (1975b) suggested the diploidizing system of *F. arundinacea* was ineffective in a hemizygous state. Two doses of the gene/locus responsible for diploid-like pairing had to be present for diploid-like behavior in MI. In a single dose, such as in a haploid *F. arundinacea* (2n = 3x = 21), up to 4.5 bivalents per cell and some trivalents are formed from homoeologous chromosomes (Sleper, 1985). This also indicated that the chromosomes of the three component genomes of *F. arundinacea* display sufficient homology to pair.

Despite the high pairing affinity, the parental genomes of *F. arundinacea* can be discriminated by genomic in situ hybridization (GISH) using total genomic DNA of the parents as probes (Humphreys et al., 1995). Since the genomes of *Lolium* can be easily discriminated from those of fescues, it is now possible to monitor the meiotic behavior of individual genomes both in *F. arundinacea* itself as well as in its hybrids with *Lolium* sp. Improved knowledge of chromosome pairing affinity of the different genomes could permit designing more effective methods for intergeneric introgressions with the aim to develop superior grass cultivars. In this study, GISH was employed to study the nature of diploid-like pairing systems in *F. arundinacea,* its progenitors *F. pratensis* and *F. glaucescens,* and two intergeneric hybrids *L. multiflorum* (2x) × *F. arundinacea* (6x) and *L. multiflorum* (4x) × *F. glaucescens* (4x), focusing specifically on homoeologous chromosome pairing and recombination of different component genomes.

Material and methods

Plant material

Seed samples or plants of tetraploid *Festuca arundinacea* ecotype (2n = 4x = 28), hexaploid *F. arundinacea* cv. 'Kora' (2n = 6x = 42), diploid *F. pratensis* cv. 'Laura' (2n = 2x = 14 and 2n = 2x = 14 + 1B), autotetraploid *F. pratensis* cv. 'Patra' (2n = 4x = 28), and F1 hybrids *L. multiflorum* × *F. arundinacea* (2n = 4x = 28; genomic constitution LmFpFgFg′) were obtained from Dr. Vladimir Černoch (Plant Breeding Station Hladké Životice, Czech Republic). *F. glaucescens* genotype '3715' (2n = 4x = 28) and two F1 hybrids *L. multiflorum* × *F. glaucescens* (2n = 4x = 28; genomic constitution LmLmFgFg′) were kindly provided by Dr. Marc Ghesquière (INRA, Lusignan, France).

Genomic in situ hybridization (GISH)

Individual anthers, confirmed to be in metaphase I (MI) or other desired stages of meiosis, were fixed in Carnoy's solution I (3 parts absolute ethanol:1 part glacial acetic acid) at 37°C for 7 days. Meiotic chromosome spreads and genomic in situ hybridization (GISH) were done according to Masoudi-Nejad et al. (2002). Sheared total genomic DNA of *L. multiflorum* was used as blocking DNA; total genomic DNAs of *F. pratensis* and *F. glaucescens* were labeled with digoxigenin using the DIG-Nick Translation Kit (Roche Applied Science, USA) and with biotin using the Biotin-Nick Translation Kit (Roche Applied Science) according to manufacturer's instructions. The probe to blocking DNA ratio was 1:150 with minor variation. The sites of probe hybridization were detected by the anti-DIG-FITC conjugate (Roche Applied Science) and by the streptavidin-Cy3 conjugate (Amersham, USA).

Chromosomes were counterstained with 1.5 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) in the Vectashield antifade solution (Vector Laboratories, USA). Microscopic preparations were

evaluated under an Olympus AX70 microscope equipped with epifluorescence and captured using the SensiCam B/W camera. ScionImage and Adobe Photoshop software v. 6 were used for recording and processing of color pictures.

Scoring of the MI chromosome pairing configurations

Chromosome pairing was scored in meiotic metaphase I; in case of the *L. multiflorum* × *F. arundinacea* hybrids, other meiotic stages (pachytene, diplotene, and anaphase I) were also observed. Whenever possible, 50 pollen mother cells (PMCs) were scored for each plant studied. The standard measure of chromosome pairing was 'arms paired per chromosome' (here abbreviated as appc): a chromosome in a ring bivalent has both arms paired (appc = 2.0), in a rod bivalent only one arm is paired (appc = 1.0), and no arms are paired in a univalent (appc = 0.0).

Six pachytene/diplotene PMCs of F1 *L. multiflorum* × *F. arundinacea* hybrids (2n = 4x = 28; LmFpFgFg′) were analyzed in detail. The length of synapsed parts of chromosomes was measured using the ScionImage software and expressed in percent of total length of a chromosome complement.

Results

Chromosome pairing at metaphase I

Festuca arundinacea. Among 150 pollen mother cells (PMCs) of three hexaploid plants of *F. arundinacea* analyzed at metaphase I, diploid-like pairing predominated, with the average of 20.75 bivalents and 0.47 univalents per PMC (Fig. 1f, g). Only in two PMCs a trivalent formed by two chromosomes of *F. pratensis* and one of *F. glaucescens* was present. No quadrivalents or higher-order configurations were observed. Chromosomes of *F. pratensis* had lower pairing indices (1.72 ± 0.18 appc) than those of *F. glaucescens* (1.82 ± 0.11 appc).

Based on the hybridization patterns observed after GISH, tetraploid *F. arundinacea* (2n = 4x = 28) consists of 14 chromosomes of *F. pratensis* and 14 chromosomes of *F. glaucescens* (Fig. 1h, Table 1). The two genomes of *F. glaucescens,* Fg and Fg′, could not be discriminated by GISH, and it was not clear whether the *F. glaucescens* genome comprised a mixture of Fg and Fg′ or just one of two genomes. In the analyzed plant, quadrivalents were formed with high frequency (2.04 per PMC). These involved pairs of Fp and Fg chromosomes and, consequently, homoeologous pairing was high.

Festuca pratensis. Five diploid plants of *F. pratensis* analyzed in this study exhibited a high frequency of bivalent pairing (mean of 6.94 per PMC), with the bivalents being mostly of the ring type; hence the chromosome arm pairing frequency was high (on average 1.88 ± 0.14 appc). One of the analyzed *F. pratensis* plants had a single B chromosome, and the overall chromosome pairing in this plant was significantly lower (1.52 ± 0.22 appc; Fig. 1a) than in any of the four plants without a B chromosome present (Fig. 1b).

In a tetraploid *F. pratensis,* chromosome pairing was quite irregular (Fig. 1c), ranging from univalents (on average 1.33 per PMC) through bivalents (on average 5.04 per PMC) to trivalents and quadrivalents (on average 0.37 and 3.87 per PMC, respectively).

Festuca glaucescens. Tetraploid *F. glaucescens* showed a strictly diploid-like chromosome pairing with the average of 13.85 bivalents per PMC, no multivalents, and a low frequency of univalents (on average 0.31 per PMC). However, most bivalents formed were rods and the overall arm pairing frequency (1.31 ± 0.16 appc) was considerably lower than in *F. pratensis* (see Fig. 1d).

Hybrids Lolium multiflorum (2x) × *Festuca arundinacea (6x).* In four plants of tetraploid F1 hybrids *L. multiflorum* × *F. arundinacea* (LmFpFgFg′) the overall pairing frequency was 1.68 ± 0.17 appc (Fig. 1i). Still, univalent frequency was high: univalents were present in 62% PMCs, with an average of 2.27 univalents per PMC. Among these, 61% were of *F. glaucescens,* 28% of *L. multiflorum*, and 11% of *F. pratensis* origin. Bivalents were formed at a frequency of 6.15 per PMC on average, and multivalents were common (3.33 quadrivalents and 0.31 trivalents per PMC). Given the genome ratio of 2:1:1 (Fg: Lm:Fp), *F. pratensis* chromosomes appeared to pair with the highest frequency and *F. glaucescens* with the lowest. The multicolor GISH indicated the following ranking of chromosome affinities: Lm-Fp > Fg-Fg > Fp-Fg > Lm-Fg with the provision that the Fg and Fg′ genomes could not be differentiated (Table 2).

Hybrids L. multiflorum (4x) × *F. glaucescens (4x).* In tetraploid F1 hybrids *L. multiflorum* × *F. glaucescens* (LmLmFgFg′), the overall pairing frequency was lower than in the *L. multiflorum* × *F. arundinacea* hybrids (1.46 ± 0.25 appc in *L. multiflorum* × *F. glaucescens* vs. 1.68 ± 0.17 appc in *L. multiflorum* × *F. arundinacea*), mainly because of a high frequency of univalents (Fig. 1e). However, the overall pairing frequency was still higher than in tetraploid *F. glaucescen*s (1.31 ± 0.16 appc). Multivalent pairing was present but at much lower frequency than that in the *L. multiflorum* × *F. arundinacea* hybrids (Table 1). The frequency of homoeologous pairing between *L. multiflorum* and *F. glaucescens* chromosomes was relatively high (ca. 20% of all pairing).

Table 1. Chromosome pairing at meiotic metaphase I in *F. pratensis, F. glaucescens, F. arundinacea,* and intergeneric *Lolium* × *Festuca* hybrids

Genotype	Chromosome number and genomic constitution[a]	Total no. of plants/PMCs analyzed[b]	Chromosome configurations per PMC, mean (range)				Appc[c]
			I	II	III	IV	
F. pratensis	2n = 2x = 14; FpFp	4/200	0.13 (0–2)	6.94 (6–7)	–	–	1.88 ± 0.28
F. pratensis	2n = 2x = 14+1B; FpFp	1/50	0.40 (0–2)	6.80 (6–7)	–	–	1.52 ± 0.22
F. pratensis	2n = 4x = 28; FpFpFpFp	4/200	1.33 (0–9)	5.04 (0–12)	0.37 (0–2)	3.87 (1–7)	1.66 ± 0.20
F. glaucescens	2n = 4x = 28; FgFgFg′Fg′	3/150	0.31 (0–4)	13.85 (12–14)	–	–	1.31 ± 0.16
F. arundinacea	2n = 4x = 28; FpFpFgFg′	1/50	2.16 (0–7)	8.48 (1–14)	0.24 (0–2)	2.04 (0–5)	1.49 ± 0.16
F. arundinacea	2n = 6x = 42; FpFpFgFgFg′Fg′	3/150	0.47 (0–4)	20.75 (19– 21)	0.01 (0–1)	–	1.79 ± 0.11
L. multiflorum × *F. glaucescens*	2n = 4x = 28; LmLmFgFg′	2/100	2.62 (0–10)	10.06 (4–14)	0.26 (0–2)	1.12 (0–3)	1.46 ± 0.25
L. multiflorum × *F. arundinacea*	2n = 4x = 28; LmFpFgFg′	4/100	1.45 (0–8)	6.15 (0–13)	0.31 (0–3)	3.33 (0–7)	1.68 ± 0.17

[a] Lm: *L. multiflorum,* Fg: *F. glaucescens,* Fp: *F. pratensis.*
[b] PMC: pollen mother cell.
[c] Arms paired per chromosome (mean ± standard deviation).

Table 2. Homologous and homoeologous pairing between chromosomes of individual genomes in *F. arundinacea* and intergeneric hybrids *L. multiflorum* (4x) × *F. glaucescens* (4x) and *L. multiflorum* (2x) × *F. arundinacea* (6x)

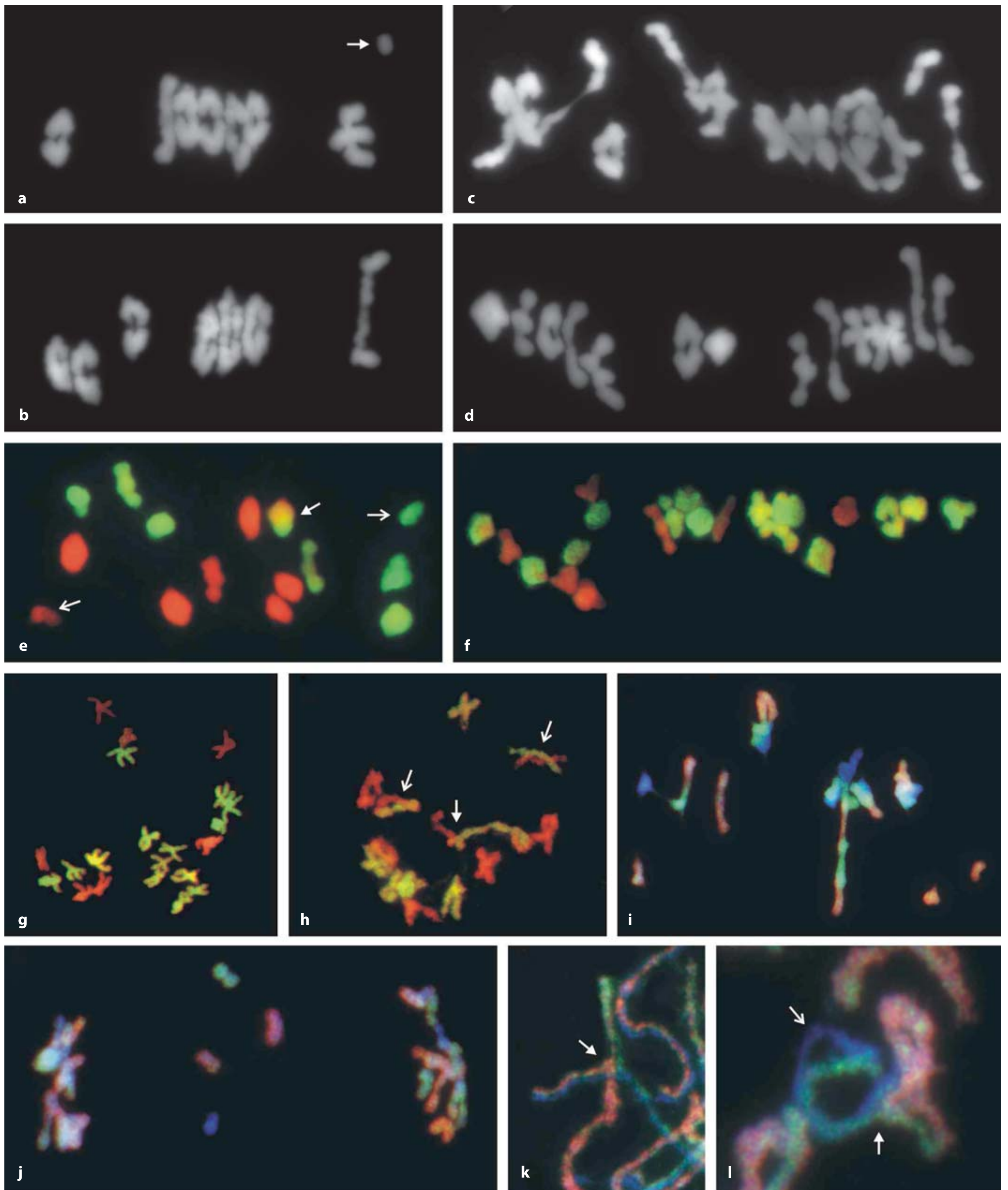
Genotype	Chromosome pairing between and within individual genomes (mean ± standard deviation per PMC)					
	Lm-Lm	Fg-Fg	Fp-Fp	Lm-Fg	Lm-Fp	Fg-Fp
F. arundinacea (4x)	–	7.44 ± 1.96	8.26 ± 1.60	–	–	5.12 ± 2.50
F. arundinacea (6x)	–	25.55 ± 1.52	12.04 ± 1.24	–	–	0.01 ± 0.11
L. multiflorum × *F. glaucescens*	8.54 ± 2.44	7.83 ± 2.32	–	4.09 ± 2.53	–	–
L. multiflorum × *F. arundinacea*	–	6.57 ± 1.74	–	4.37 ± 1.75	7.16 ± 1.59	5.39 ± 1.68

Lm: *L. multiflorum*, Fg: *F. glaucescens*, Fp: *F. pratensis.*

Chromosome behavior at pachytene, diplotene, and anaphase I

Behavior of individual genomes in *L. multiflorum* (2x) × *F. arundinacea* (6x) hybrids was also observed in pachytene and diplotene, and the separating chromosomes were observed at anaphase I (Fig. 1j). In six of the pachytene/diplotene PMCs observed in detail, the highest extent of synapsis was found between chromosomes of *L. multiflorum* and *F. glaucescens* (28% of all chromosome length was in synapsis of these two genomes). A lower extent of synapsis was recorded between chromosomes of *F. pratensis* and *F. glaucescens* (25%), of *L. multiflorum* and *F. pratensis* (20%), and of *F. glaucescens* subgenomes (20%). Approximately 7% of the length of all chromosomes was left unsynapsed. Among the PMCs studied in detail, several instances of the pairing partner exchange were present (Fig. 1k, l), as well as synapsis of homoeologous chromosomes, where part of the chromosome pair is left unsynapsed (Fig. 1l).

In anaphase I, lagging chromosomes were present in 61% of PMCs. Among those, 68% belonged to *F. glaucescens* genomes, 28% were of *L. multiflorum*, and 4% were of *F. pratensis.* This represents a deviation from the expected frequencies based on genome proportions (2 Fg: 1 Lm:1 Fp), but appears to coarsely parallel the pairing frequencies of individual genomes (see above).



Discussion

A diploid-like pairing system, which is common in amphiploids, prevents meiotic irregularities and improves the efficiency of gamete production. It is likely that bread wheat would have never achieved its status of the largest commodity if it wasn't for its *Ph1* chromosome pairing system. The wheat system is the most studied and consists of several loci. The two most important genes are *Ph1* and *Ph2* located on chromosomes 5BL and 3DS, respectively (Riley and Chapman, 1958; Sears and Okamoto, 1958; Sears, 1977). Some diploidizing system seems to be present in polyploid fescues (e.g., *F. arundinacea, F. glaucescens,* and *F. gigantea*), but its nature is not known. In hexaploid *F. arundinacea* a breakthrough of a diploid-like pairing system was observed only exceptionally. In this study, we detected only two trivalents among 150 PMCs, and Kleijer (1984) and Zwierzykowski (1980) reported similarly a low frequency of trivalents and quadrivalents.

Jauhar (1993) indicated that the action of a diploid-like pairing system in *F. arundinacea* can be manipulated by crossing different ecotypes, suggesting the effect of the genotype. Based on the analysis of MI chromosome pairing in hybrids *(L. multiflorum × F. apenina) × F. arundinacea,* Thomas et al. (1983) proposed that there were at least two genes with additive effect controlling diploid-like pairing behavior. By screening monosomic lines of *F. arundinacea,* Jauhar (1975b) found one line where the diploid-like pairing system was disrupted, presumably because the pairing system was located on a missing chromosome. Unfortunately, that line was lost over time. However, Kleijer and Morel (1984) considered the disruption of a diploid-like pairing system in one of the monosomic plants of Jauhar (1975b) as a consequence of normal variation among plants.

As mentioned earlier, the fescue system is probably haploinsufficient. The haploinsufficiency is rare among plant genera, but it is not an exception. From breeders' point of view, the hemizygous-ineffective nature of the fescue system is advantageous as it makes intergeneric introgressions easier to accomplish. While in wide hybrids of wheat the dominant *Ph1* locus prevents pairing of all except the most closely related chromosomes, homoeologous pairing and recombination in F1 hybrids of *F. arundinacea* with *L. multiflorum* or *L. perenne* is fre-

Fig. 1. The analysis of chromosome pairing during meiosis in (**a**, **b**) diploid *F. pratensis*, (**c**) tetraploid *F. pratensis*, (**d**) tetraploid *F. glaucescens*, (**f**, **g**) hexaploid *F. arundinacea*, (**h**) tetraploid *F. arundinacea*, and (**e**) tetraploid F1 hybrids *L. multiflorum × F. glaucescens* and (**i**–**l**) *L. multiflorum × F. arundinacea*. The preparations were either stained by DAPI (**a**–**d**) or subjected to GISH and counterstained by DAPI (**e**–**l**). (**a**) Chromosome pairing in diploid *F. pratensis* (2n = 2x = 14 + 1B) with one B chromosome on the periphery (arrow) in metaphase I: 5 ring and 2 rod bivalents. (**b**) Chromosome pairing in diploid *F. pratensis* (2n = 2x = 14) in metaphase I: 6 ring and 1 rod bivalents. (**c**) Chromosome pairing in tetraploid *F. pratensis* cv. 'Patra' (2n = 4x = 28) in metaphase I: 4 quadrivalents (2 ring and 2 chain), and 6 bivalents (4 ring and 2 rod). (**d**) Chromosome pairing in tetraploid *F. glaucescens* (2n = 4x = 28) in metaphase I with 14 bivalents (4 ring and 10 rod). (**e**) Chromosome pairing in F1 hybrid of *L. multiflorum × F. glaucescens* (2n = 4x = 28). Genomic DNA of *F. glaucescens* was labeled with digoxigenin and detected by anti-DIG-FITC (green color); genomic DNA of *L. multiflorum* was used as blocking DNA (no label). The chromosomes were counterstained by DAPI (red pseudocolor). Note the homoeologous pairing in the ring bivalent (arrow) and univalents of both genera (open arrows). Chromosome pairing in hexaploid (**f**, **g**) and tetraploid (**h**) *F. arundinacea* (2n = 6x = 42 and 2n = 4x = 28) during metaphase I (**f**) and diakinesis (**g**, **h**) of meiosis. Genomic DNA of *F. glaucescens* was labeled with digoxigenin and detected by anti-DIG-FITC (green color) and genomic DNA of *F. pratensis* was used as blocking DNA (no label). The chromosomes were counterstained by DAPI (red pseudocolor). Note that 21 bivalents consist of strictly homologous pairs in hexaploid plants and formation of homoeologous bivalents (open arrows) and quadrivalent (arrow) in a tetraploid plant. Chromosome pairing in tetraploid F1 hybrid of *L. multiflorum × F. arundinacea* (2n = 4x = 28; LmFpFgFg′) during metaphase I (**i**), anaphase I (**j**), late pachytene (**k**), and diplotene (**l**). Genomic DNA of *F. pratensis* was labeled with digoxigenin and detected by anti-DIG-FITC (green color), genomic DNA of *F. glaucescens* was labeled with biotin and detected by streptavidin Cy-3 (red color) and sheared genomic DNA of *L. multiflorum* was used as blocking DNA (no label). The chromosomes were counterstained by DAPI (blue color). Note the high frequency of homoeologous pairing during metaphase I, where five homoeologous quadruplets can be seen (**i**). In anaphase I, lagged chromosomes of all component genomes are shown (**j**). During prophase I stages (**k**, **l**), changing of pairing partner (solid arrows) and non-complete homoeologous pairing (open arrow) can be seen.

quent. This is of extremely high value for breeding, especially if it is possible to stabilize the hybrid genome with introgressed agronomically interesting traits. This can be achieved either by amphiploidization or backcrossing.

Surprisingly, doubling the chromosome number and generating new amphiploids does not necessarily restore the regular bivalent pairing. In tetraploid *L. multiflorum* (2x) × *F. arundinacea* (6x) F1 hybrids, where each genome was present in one copy, chromosome pairing in metaphase I was high and, by definition, all of it must have been homoeologous (Jauhar, 1975a). In allooctoploids obtained after colchicine treatment from tetraploid hybrids of *L. multiflorum* (2x) × *F. arundinacea* (6x), Zwierzykowski (1980) observed frequent multivalents (0.95–1.27 trivalents, 0.30–0.80 quadrivalents, 0.10–1.20 pentavalents, and 0–0.05 hexavalents per PMC) despite the fact that all genomes were present in two copies. Similarly, Morgan et al. (1988) detected formation of multivalents in amphiploids of *L. multiflorum* × *F. gigantea* indicating that the action of a diploid-like pairing system was not restored completely. These results clearly show that the diploid-like pairing system is not restored in hybrids by amphiploidy.

At present, there is no plausible explanation for this phenomenon; perhaps the system is leaky and/or there are interactions of this system with chromosome pairing/recognition mechanisms in the diploid parents. This is not necessarily an odd expectation. For example in wheat-rye hybrids, rye chromosome 5 is capable of suppressing, to some extent, the normal *Ph1* allele in wheat and thus leading to some homoeologous pairing and recombination (Riley et al., 1973). In this context, Kleijer and Morel (1984) speculated that non-restoring of a diploid-like pairing was associated with the presence of the *Lolium* genome, which can suppress, to some extent, the action of the system. We can deduce that an amphiploidization strategy is presumably not the effective option to stabilize genome constitution, restore regular meiosis, and hence produce highly fertile hybrid genotypes. To achieve this goal, several rounds of backcrosses seem to have a higher potential. This notion is supported by the fact that until now all commercially used cultivars of tall fescue × ryegrass hybrids were produced by backcrossing.

The genomic location of the pairing control gene/genes in *F. arundinacea* is unknown. As *F. arundinacea* comprises genomes of two progenitor species, *F. pratensis* and *F. glaucescens,* the pattern of pairing in hybrids of *F. arundinacea* with these progenitors ought to indicate if the pairing control system evolved after *F. arundinacea* was formed or if it was inherited from one or both progenitors. Nilsson (1940) reported mostly 7 bivalents and 14 univalents in MI of tetraploid F1 hybrids of *F. pratensis* (2x) × *F. arundinacea* (6x), suggesting that, if the diploid-like pairing system was hemizygous ineffective, the locus for the system had to reside in the *F. pratensis* genome. In the tetraploid hybrid FpFpFgFg′ only the *F. pratensis* genome is present in two copies. However, Myers and Hill (1947) and Malik and Thomas (1967) observed more than seven bivalents per cell as well as multivalents (tri- and quadrivalents) in the hybrids with the same chromosome constitution. This indicates a location of a pairing control system on one of the *F. glaucescens* subgenomes.

In this study, we report on the presence of multivalents in MI of the induced tetraploid *F. pratensis,* while in tetraploid *F. glaucescens* pairing was restricted to bivalents. Given the extreme chromosome similarity in autotetraploids (pairs of chromosomes originate from sister chromatids), one can reasonably expect to see multivalents, whether a diploidizing system is present or not. On the other hand, in hybrids obtained after crossing hexaploid *F. arundinacea* with diploids, pairing of the two *F. glaucescens* genomes was frequent, suggesting their considerable affinity when the diploidizing system is ineffective (here by hemizygosity). Therefore, we are inclined to believe that the locus for the diploid-like pairing system in *F. arundinacea* is located in the *F. glaucescens* genome rather than in *F. pratensis.*

As mentioned above, the extent of homoeologous pairing in interspecific hybrids should be considered during breeding of hybrid cultivars. Before the advent of molecular cytogenetics, discrimination of individual genomes was not possible, and the extent of homoeologous pairing and recombination in wide hybrids of *F. arundinacea* could only be estimated based on indirect evidence. The most common approach was the screening for the occurrence of multivalents during MI in polyploid hybrids (Malik and Thomas, 1967; Jauhar, 1975a; Thomas et al., 1983; Ghesquière et al., 1993). For example, in F1 hybrids *L. multiflorum* (2x) × *F. arundinacea* (6x) the extent of homoeologous chromosome pairing during meiosis was assessed according to the presence of multivalents (Crowder, 1953; Zwierzykowski, 1980; Thomas et al., 1983; Kleijer, 1984; Eizenga and Buckner, 1986). These early studies suggested that chromosomes of all three genomes involved in hybrids *(L. multiflorum, F. pratensis,* and *F. glaucescens)* could pair with each other, but the actual frequencies could not be gauged by the conventional cytological techniques.

The availability of GISH permitted reassessment of the earlier results and to evaluate in more detail chromosome pairing and recombination in wide hybrids of grasses. Thus, Morgan et al. (2001) revealed a predominance of homologous pairing between *Lolium* chromosomes (5:1 ratio of homologous vs. homoeologous pairing) in hybrids between the tetraploid F1 hybrid *L. multiflorum* × *F. glaucescens* (LmLmFgFg′) and diploid *L. multiflorum*. In this study, the ratio of homologous vs. homoeologous pairing was similar. Even a lower frequency of homoeologous pairing was observed in the same hybrid combination by Humphreys et al. (2005), who reported a 9:1 ratio of homologous vs. homoeologous pairing. Similarly, a 9:1 ratio of homologous vs. homoeologous pairing was detected in tetraploid F1 *F. pratensis* × *L. perenne* hybrids (Zwierzykowski et al., 2008). An even lower frequency of homoeologous association (14:1 ratio of homologous vs. homoeologous pairing) was reported in our previous study in tetraploid F1 *L. multiflorum* × *F. pratensis* hybrids (Kopecký et al., 2008).

These results indicate that if the chromosomes of both parental species are present in an alloploid in a disomic constitution, there is a preferential homologous pairing; however, homoeologous associations appear in some frequency. Our recent study on pairing behavior of individual chromosomes of *F. pratensis* substituted in monosomic and disomic constitution into tetraploid *L. multiflorum* (Kopecký et al., 2008) confirmed these observations. With two pairs of homologues present, one of *Festuca* and one of *Lolium,* there was a clear preference for homologous pairing for each of the chromosomes tested. However, with only a single chromosome of *Festuca* and three of *L. multiflorum* present, the single *F. pratensis* chromosome, facing the choice of pairing homoeologously or not pairing at all, always paired with its *Lolium* homoeologue(s), with the same frequency as homologues did.

The present study on F1 hybrids of *L. multiflorum* × *F. arundinacea* shows that if chromosomes of the parental species are present in monosomic constitution, homoeologous multivalent formation is high and even homoeologous bivalents are frequently formed. When considering our results on MI pairing and the occurrence of lagging chromosomes, *L. multiflorum* and *F. pratensis* genomes have the highest affinity, but the differences in homoeologous affinities of all genomes are small. The analysis of homoeologous chromosome behavior during prophase I suggests slightly different genome affinities, with *L. multiflorum* and *F. glaucescens* exhibiting the highest frequency of synapsis. However, the number of analyzed PMCs was too low to assess the genome relationship.

In this work we report on small differences in the frequency of chromosome pairing between component genomes in *Festuca* × *Lolium* hybrids. This indicates that introgression of any specific chromosome segment from one genome to another is possible in all genome combinations and the decision which genomes recombine can be influenced by carefully selecting the crossing partners. This could be the first step on the way towards chromosome engineering in forage grasses similar in scope and precision to that currently practiced in wheat, where not only the donor but also the recipient chromosomes can be selected and successfully manipulated.

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