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Alien introgression in the grasses *Lolium perenne* (perennial ryegrass) and *Festuca pratensis* (meadow fescue): the development of seven monosomic substitution lines and their molecular and cytological characterization

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• *Background and Aims* To address the issues associated with food security, environmental change and bioenergy in the context of crop plants, the production, identification and evaluation of novel plant phenotypes is fundamental. One of the major routes to this end will be wide hybridization and introgression breeding. The transfer of chromosomes and chromosome segments between related species (chromosome engineering or alien introgression) also provides an important resource for determining the genetic control of target traits. However, the realization of the full potential of chromosome engineering has previously been hampered by the inability to identify and characterize interspecific introgressions accurately.
• *Methods* Seven monosomic substitution lines have been generated comprising *Festuca pratensis* as the donor species and *Lolium perenne* as the recipient. Each of the seven lines has a different *L. perenne* chromosome replaced by the homoeologous *F. pratensis* chromosome (13 *L. perenne* + 1 *F. pratensis* chromosome). Molecular markers and genomic *in situ* hybridization (GISH) were used to assign the *F. pratensis* chromosomes introgressed in each of the monosomic substitutions to a specific linkage group. Cytological observations were also carried out on metaphase I of meiosis in each of the substitution lines.
• *Results* A significant level of synteny was found at the macro-level between *L. perenne* and *F. pratensis*. The observations at metaphase I revealed the presence of a low level of interspecific chromosomal translocations between these species.
• *Discussion* The isolation of the seven monosomic substitution lines provides a resource for dissecting the genetic control of important traits and for gene isolation. Parallels between the *L. perenne/F. pratensis* system and the Pooideae cereals such as wheat, barley, rye, oats and the model grass *Brachypodium distachyon* present opportunities for a comparison across the species in terms of genotype and phenotype.

Key words: *Lolium perenne*, *Festuca pratensis*, chromosome engineering, molecular markers, genomic *in situ* hybridization, recombination, comparative genomics, Pooideae, genetics, introgression.

INTRODUCTION

The identification and isolation of the genes via forward genetic approaches, i.e. genetic mapping, chromosome landing, etc., requires the presence of sufficient phenotypic variation for the trait in question. In inbreeding species such as wheat, very little genetic variation exists in adapted genotypes. However, wild hexaploid landraces and wild and distant relatives (alien species) (e.g. rye and *Thinopyrum bessarabicum*) provide a key source of variation for target traits. In outbreeding highly heterozygous crops such as forage grass, considerable variation still exists in elite plant material for many traits. *Lolium perenne*, the major source of forage in temperate regions of the world, shows considerable variation for quality traits such as digestibility, establishment, and spring and winter growth. However, *L. perenne* shows less variation for traits which are of importance for resistance to abiotic stresses, e.g. drought tolerance, winter hardiness, water use efficiency, root development and persistency (Kosmala *et al.*, 2007). Considerable genetic variation for these traits does exist within closely related alien species such as *Festuca pratensis*, *F. glaucescens*, *F. arundinacea*, *F. maire* and *F. atlantigena*, with which *Lolium* species such as *L. perenne* and *L. multiflorum* can be readily hybridized (King *et al.*, 2007*b*).

Thus wild and cultivated relatives (alien species) of crop species provide a wealth of genetic variation for potentially all characters of strategic and fundamental importance, e.g. yield, climate change and the environment, recombination frequency and distribution, and perenniality. This genetic variation from alien species can be exploited both directly, i.e. target genes can be transferred into a crop species leading to the development of superior varieties, and indirectly, i.e. genes from alien species can be used to determine the genetic control of target traits and the pathways involved in their expression (Armstead *et al.*, 2006*a*, *b*, 2007; Sandve *et al.*, 2008).

Lolium perenne and *F. pratensis* can be readily hybridized to form interspecific hybrids and are indeed found as natural hybrids through North West Europe (Borrill, 1975; Humphreys *et al.*, 2003). At meiosis the chromosomes of these species recombine at high frequency, yielding progeny that contain *L. perenne/F. pratensis* recombinant chromosomes that can be characterized using genetic markers (King *et al.*, 2002*a*, *b*, 2007*a*, *b*; Kopecky *et al.*, 2009) and also genomic *in situ* hybridization (GISH; King *et al.*, 1998*a*, *b*, 2007*a*). Here we report the isolation of seven *L. perenne/F. pratensis* monosomic substitution lines, i.e. in each of the lines one of the seven chromosomes of *L. perenne* has been replaced with its *F. pratensis* homoeologue (13 *L. perenne* + 1 *F. pratensis* chromosomes). The potential for the exploitation of these monosomic substitution lines is discussed.

MATERIALS AND METHODS

Monosomic substitution lines of *Lolium perenne* × *Festuca pratensis* (Fig. 1) were identified from an initial population of BC_1 individuals using a combination of GISH, amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) screens, in a background of detailed karyotypic knowledge, as described by King *et al.* (1998*a*, 2002*a*, *b*). Briefly GISH was used to identify genotypes which contained whole chromosome introgressions. AFLP templates were then prepared from this core set of genotypes and these were screened with a series of selective primer pairs: (*a*) to confirm the *F. pratensis* introgression by identifying *Festuca*-specific AFLP bands segregating in the target genotypes and (*b*) to identify which introgressions were derived from the same and which from different *F. pratensis* chromosome introgressions. Genetically mapped and chromosome-specific RFLPs and, subsequently, sequence tagged site (STS) and simple sequence repeat (SSR) markers were used to associate the introgressed *F. pratensis* chromosomes with previously identified linkage groups (Table 1). An alternative strategy of screening BC_1 genotypes with chromosome-specific markers prior to GISH evaluation was also used to identify the last two monosomic substitutions with an expanded BC_1 family.

The seven monosomic substitution lines are perennial outbreeders and therefore have to be maintained as clones. Multiplying each genotype is done simply by tillering down and potting on. In this way it is possible to maintain the plants indefinitely.

Feulgen and GISH analyses were performed on pollen mother cells (PMCs) from each of the monosomic substitution lines at metaphase I of meiosis (Fig. 2) to observe the meiotic behaviour of the *F. pratensis* chromosomes (after King *et al.*, 1998*b*).

RESULTS

Out of 161 BC_1 genotypes screened with GISH, five genotypes appeared to carry a single, whole *F. pratensis* chromosome introgression (i.e. 1 *F. pratensis* + 13 *L. perenne* chromsomes). AFLP analysis identified unique *F. pratensis*-derived AFLP bands in all five genotypes containing the *F. pratensis* whole chromosome introgressions, indicating that all five genotypes probably contained different *F. pratensis*-derived chromsomes, i.e. five of the seven possible target chromosomes, a conclusion compatible with the karyotype observations (Fig. 3). Some overlapping *F. pratensis*-derived AFLP bands were identified between two of the genotypes. Repetition of the GISH analysis indicated that one of the genotypes had a whole chromosome plus a small terminal *F. pratensis* introgressed segment which had not been detected in the initial GISH images; this small introgressed segment was subsequently removed by a further round of backcrossing. Screening of the five different genotypes containing the whole chromosome introgressions with RFLP and SSR markers previously mapped to defined linkage groups (King *et al.*, 2002*a*, 2008; J. King *et al.*, unpubl. res.) identified the introgressed chromosomes as being derived from C1, C2, C3 (King *et al.*, 2002*a*, *b*), C4 and C7 (Table 1). To isolate C5 and C6, a further 446 BC_1 genotypes were generated and screened with C5- and C6-specific RFLP, STS and SSR markers to identify candidate genotypes containing at least partial C5 and C6 *F. pratensis* introgressions. GISH was then used to distinguish partial from whole chromosome introgressions, and this resulted in the identification of both C5 and C6 candidate monosomic substitutions. As with C1–C4 and C7, this was confirmed by karyotype evaluation (Fig. 3) and SSR screening (Table 1).

Meiotic analyses of Feulgen-stained PMCs from the diploid *L. perenne* genotype (the recurrent crossing parent) and each of the seven substitutions were analysed. A low frequency of univalents but no multivalents were recorded in the *L. perenne* genotype, but the meiotic analysis did reveal the presence of univalents and a low frequency of multivalent formation within the substitution lines (Table 2). The mean number of chiasmata per cell in each of the monosomic substitution lines did not vary significantly from the mean number recorded for *L. perenne* ($\chi^2 = 0{\cdot}03$, $0{\cdot}13$, $0{\cdot}03$, $0{\cdot}05$, $0{\cdot}003$, $0{\cdot}01$ and $0{\cdot}003$ for substitution lines 1–7, d.f. = 1) at the 1 % level of probability. In order to determine if the introgressed *F. pratensis* chromosomes were involved in multivalent formation or the observed pairing failure, 25 PMCs per line were analysed at metaphase I of meiosis using GISH (Fig. 2). A very low frequency of multivalent formation involving the *F. pratensis* chromosome was observed, indicating the potential presence of interspecific chromosomal translocations (Table 3). The chiasma frequency within the homoeologous *L. perenne/F. pratensis* bivalent varied between $1{\cdot}25$ (substitution line 5) and $1{\cdot}9$ (substitution line 4). The chiasma frequency between *Lolium/Lolium* bivalents fell within this range, i.e. the chiasma frequency for the *L. perenne/F. pratensis* bivalent was slightly lower than the average chiasma frequency for all bivalents in substitution lines 1, 5 and 6, equal to it in substitution line 2 and higher in substitution lines 3, 4 and 7. The homoeologous *L. perenne* and *F. pratensis* chromosomes formed both rods (resulting from a single chiasmate exchange between two homoeologous chromosome arms) and rings (that resulted from two chiasmata between all four homoeologous chromosome arms; Fig. 2). Some differences were noted in the mean chiasmata per cell in Table 2 as compared with Table 3 although a χ^2 test on these differences was not significant ($\chi^2 = 1{\cdot}79$, d.f. = 6) at the 1 % level of probability.

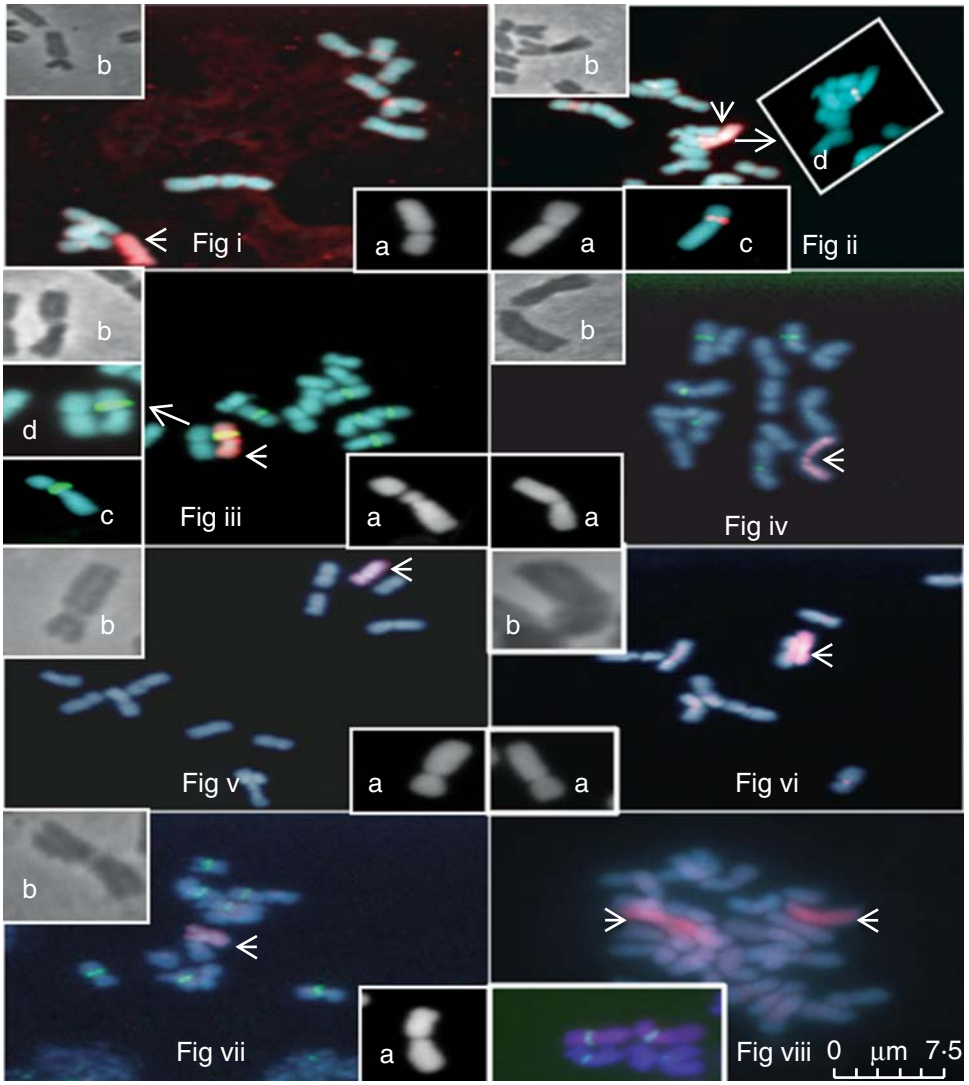

FIG. 1. Mitosis in the seven monosomic substitution lines. (Fig i–vii) Monosomic lines 1–7; the substituted chromosome is shown by an arrow. Inset images: (a) 4′,6-diamidino-2-phenylindole (DAPI)-stained image of the relevant substitution line; (b) phase contrast image of the relevant substitution line. (Fig ii) Inset images: (c) and d) show the 5s site in monosomic line 2. (Fig iii) Inset images: (c) and (d) show monosomic line 3 carrying the pta71 ribosomal site. (Fig viii) A colchicine doubled line of monosomic line 3 ($2n = 28$) carrying a disomic substitution. Inset image: (a) shows the disomic chromosome substitutions for monosomic line 3 carrying the two pta71 sites. Scale bar = 7·5 μm.

DISCUSSION

Table 1 lists the markers used for the association of the individual whole chromosome introgressions with particular chromosome designations (e.g. 1–7). While, in general, the syntenic relationships are consistent, 16 of the total 136 markers (approx. 12 %) identified different *F. pratensis* chromosomes from the one to which they had been mapped in *L. perenne* (Alm *et al.*, 2003 also noted some limited, apparent breakdown in synteny between *L. perenne* and *F. pratensis*, with approx. 9 % of the common markers mapping to non-syntenic loci). There are a number of possible explanations for this. (*a*) While most SSR markers identify only a single locus, it is not infrequent that they can identify more than one locus on the same or on different linkage groups. Therefore, the locus tagged by a particular SSR in this introgression study may be different from the locus mapped in a different mapping population. (*b*) Small sequence differences between the SSR priming sites present in *L. perenne* and *F. pratensis* may lead to a particular SSR tagging different loci in the two species. (*c*) The conservation of synteny between *L. perenne* and *F. pratensis* chromosomes is not complete, i.e. there are some genomic rearrangements between the two species. The first two of these explanations really reflect limitations in marker technology, e.g. are the markers identifying the same loci? While this study cannot discriminate directly between these three explanations, work currently being undertaken on mapping populations produced from the monosomic substitution lines would suggest that any breakdown in synteny would be limited to disruptions to micro-colinearity rather than macro-colinearity. However, it is worth noting that for C4, approx. 25 % of the markers that tagged the *F. pratensis*-derived introgression had been

TABLE 1. *Markers used to assign chromosome identities to* F. pratensis *whole-chromosome introgression lines*

Line 1			Line 2			Line 3			Line 4			Line 5			Line 6			Line 7		
Marker	C*	S†	Marker	C	S	Marker	C	S	Marker	C	S	Marker	C	S	Marker	C	S	Marker	C	S
Rv0003	1	A	Rv0062	2	A,B	Rv0753	3	A	Rv0161	4	F	Rv0054	5	A	Rv0067	6	B	Rv0009	7	A
Rv0033	1	A,B	Rv0226	2	A	Rv0774	3	G	Rv0380	4	A,B	Rv0184	5	A	Rv0144	6	A	Rv0011	7	A
Rv0137	1	A	Rv1008	2	A	Rv0863	3	A	Rv0454	4	A,B	Rv0250	5	A,B	Rv0297	6	A	Rv0264	7	A,B
Rv0165	1	A	Rv1031	2	A	Rv1046	3	A,B	Rv0650	4	A	Rv0342	5	A	Rv0609	6	A	Rv0333	7	A
Rv0301	1	A,B	Rv1068	2	A	Rv1131	3	A,B	Rv0922	4	A	Rv0950	5	A	Rv0641	6	A,B	Rv0817	7	A
Rv0367	1	A	Rv1164	2	A	Rv1172	3	A	Rv0966	4	A,B	Rv1112	5	A,B	Rv0985	6	A	Rv1171	7	A,B
Rv0394	1	A	Rv1239	2	A	Rv1390	3	F	Rv1053	4	A	Rv1139	5	A	Rv1036	6	A	Rv1254	7	A
Rv0624	1	A	Rv1268	2	A	Rv1439	3	A	Rv1056	4	A	Rv1307	5	A	Rv1093	6	F	Rv1408	7	A
Rv1391	1	A,B	Rv1269	2	A	B3B8	3	F	Rv1127	4	G	CDO127	5	H	Rv1208	6	A,B	Rv1411	7	A,B
17ga1	1	A	LpSSRH01A01	2	C	Rv0677	3	A	Rv1153	4	A	Rv0157	3,7	A	Rv1266	6	A	08ga1	7	A
LpACT15H3	1	E	Rv0188	2	A,B	CDO345	3	H,I,J	Rv1190	4	A,B	Rv0752	4	A	LpACT14C9	6	E	LpACT13F9	7	E
LpACA11H9	1	F	CDO1417	2	H	C949	3	H,I	Rv1295	4	A	Rv0809	4	A	LpACA24B4	6	F	LpACA11D4	7	F
PR8	1	F	CDO405	2	H	C250	3	H,I	LpACA8B9	4	E				NFA048	6	F	LpHCA17C6	7	E
PR25	1	F	CDO36	2	H,J	WG889	3	H,I	Rv0262	4	A				Rye014	6	B,C	B3C5	7	F
PR37	1	F	BCD855	2	H	BCD828	3	H,I	Rv0329	4	A				Rye0739		A	Rv0663	7	A
Rv1097	1	A	Rv0447	3	A	CDO455	3	H,I	Rv0954	4	A							Rv0908	7	A,B
CDO98	1	H	Rv0229	6	G	PSR370	3	H,I	Rv1089	4	A							06g10880	7	D
CDO202	1	H,J				PSR394	3	H,I,J	Rv1302	4	G							LpHCA17C6	7	F
Rv1154	2	A				CDO920	3	H,I,J	CDO122	4	H,J							BCD147	7	H
						R1613	3	H,I	CDO20	4	H							CDO545	7	H,J
						CDO328	3	H,I	Rv1087	1	A							Rv0620	6	A
						CDO460	3	H,I,J	Rv0785	1	A									
						C30	3	H,I	Rv0810	1,7	A									
						Rv0756	2	G	Rv0103	2	A									
						Rv0680	4	A	Rv1063	3	A									
									Rv0372	5	G									
									Rv0551	7	A									

* Chromosome to which the marker has been assigned by the mapping study referred to in column S.

† Mapping studies: A, Gill *et al.* (2006); B, Turner *et al.* (2008); C, Armstead *et al.* (2004); D, Armstead *et al.* (2008); E, King *et al.* (2008); F, J. King (unpubl. res.); G, G. Gill (Vialactia Biosciences, unpubl. res.); H, Armstead *et al.* (2002); J, Alm *et al.* (2003).

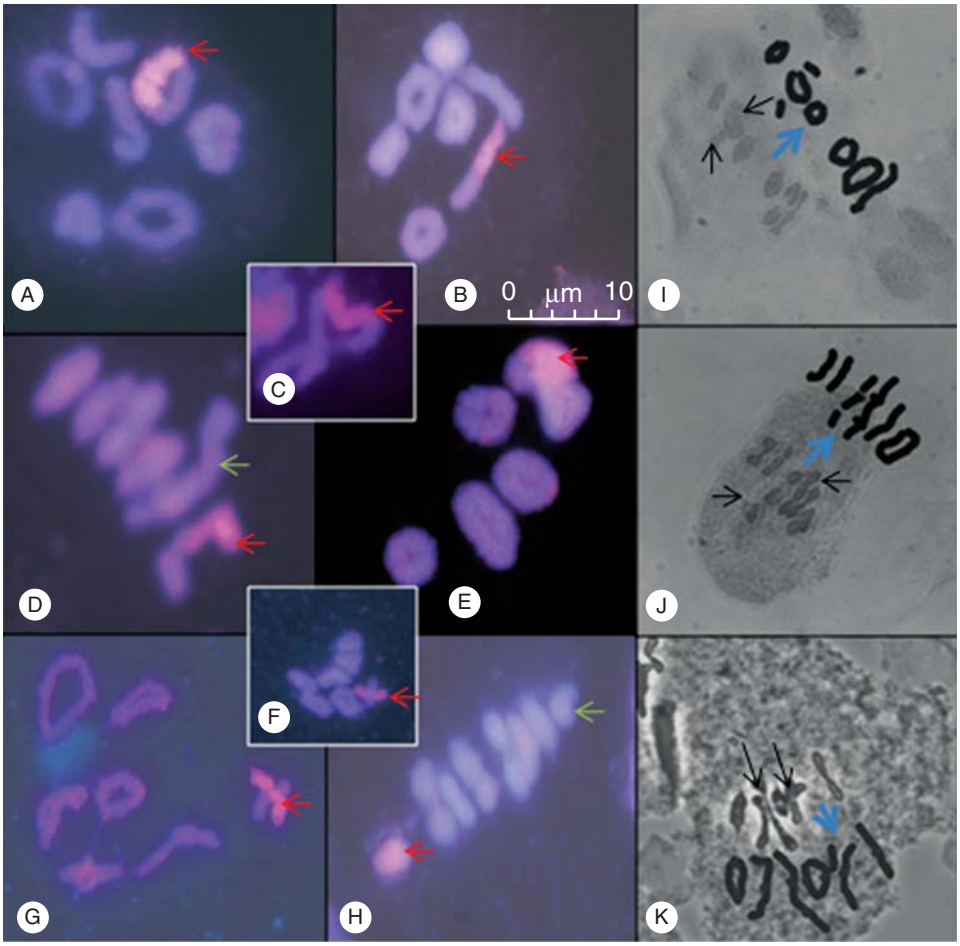

FIG. 2. The analysis of chromosome pairing during meiotic metaphase I in *L. perenne* × *F. pratensis* monosomic substitution lines. (A–H) GISH images of meiotic metaphase I with *F. pratensis* (*Fp*) chromosome shown by a red arrow. (I–K) Phase images of pairing during meiotic metaphase I and drawn representations of the meiotic configuration shown by a blue arrow. (A) Monosomic line 3 showing a ring bivalent involving the *Fp* chromosome. (B) Monosomic line 5 showing a rod bivalent involving the *Fp* chromosome. (C) Monosomic line 4 showing the *Fp* chromosome as part of a quadrivalent. (D) Monosomic line 2 with a rod bivalent, but a quadrivalent is also present involving *L. perenne* (*Lp*) chromosomes shown by the green arrow. (E) Monosomic line 2 showing the *Fp* chromosome as part of a quadrivalent. (F) Proximal chiasma in monosomic line 1. (G) Proximal chiasma in monosomic line 5. (H) Monosomic line 2 showing the *Fp* chromosome as a univalent and also an *Lp* univalent (green arrow). (I) Monosomic line 3 showing two univalents. (J) Monosomic line 4 with one trivalent and three univalents. (K) Monosomic line 5 with one trivalent and one univalent.

mapped to different linkage groups in previous *L. perenne* mapping studies. This indicates that a genomic rearrangement might be more likely for this chromosome than the others. Further evidence for a genomic rearrangement comes from the observation that the group four *F. pratensis* chromosome is occasionally involved in multivalent formation. If this were the case, the nature of the genomic rearrangement would not appear to be a simple translocation, as the seven non-syntenous markers have been previously mapped to five different linkage groups (Table 1).

Univalents and rare multivalents have occasionally been observed in Feulgen-stained preparations of PMCs from some of the seven monosomic substitution lines (Table 2). In order to determine whether the *F. pratensis* chromosomes in these lines were involved in multivalent formation, or pairing failure resulting in univalent formation, GISH analysis was performed. Multivalents involving the *F. pratensis* chromosomes and the *L. perenne* genome would have indicated a breakdown in synteny between the two species at the macro-level. For example, in the absence of interspecific chromosomal rearrangements, only bivalents would be formed at metaphase I of meiosis, i.e. each *Festuca* chromosome shows complete synteny with its *Lolium* homoeologue. However, the involvement of a *Festuca* chromosome in a multivalent would indicate that it shows a syntenic relationship with more than one *Lolium* syntenic group. Multivalents were observed involving the *Festuca* chromosomes in substitution lines 1, 2 and 4. It therefore appears there maybe a breakdown in synteny between these chromosomes in *Lolium* and *Festuca* at the macro-level (especially as no multivalents were observed in a normal *Lolium* genotype; Table 2). However, the frequency of multivalent formation was very low, i.e. 0·04 %, indicating that the level of breakdown in synteny is low (Table 3). In addition, the presence of ring bivalents resulting from recombination involving both arms of the *L. perenne* and *F. pratensis* chromosomes indicates a relatively high level of conservation of synteny at the macro-level between the chromosomes of these species and thus confirms

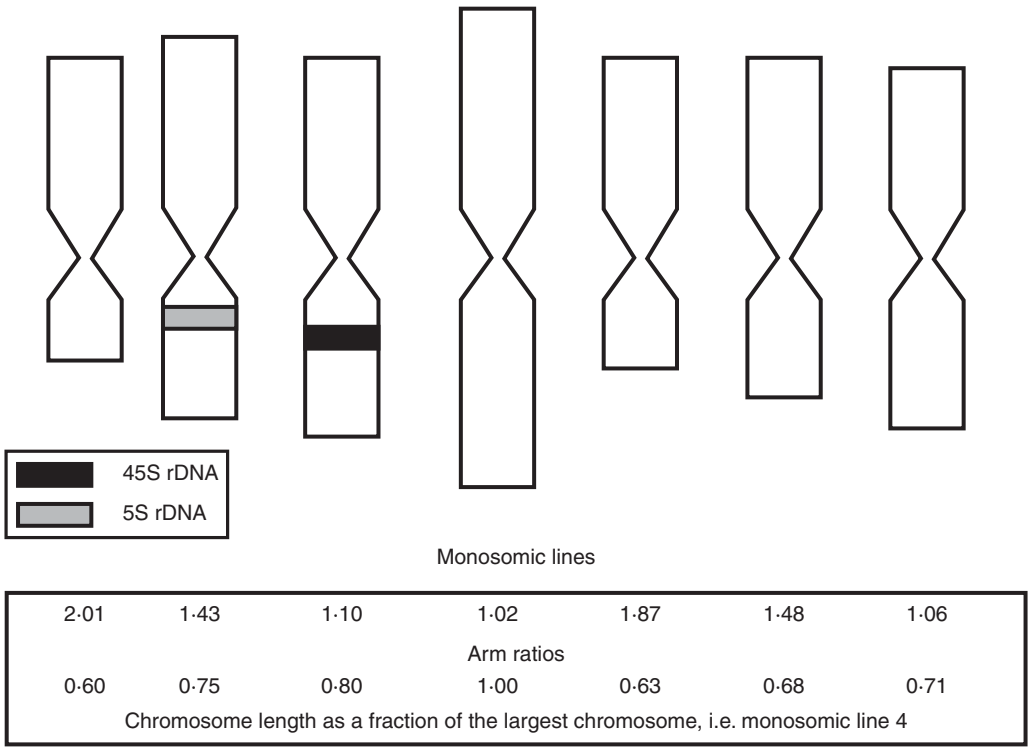

FIG. 3. The individual chromosomes of *F. pratensis* assigned to their respective Triticeae linkage group. A combination of arm ratio, chromosome length, 5S rDNA and 45S rDNA allows identification of each of the *F. pratensis* chromosomes. The chromosomes are drawn to scale.

TABLE 2. *Frequency of univalents, bivalents and multivalents in 50 Feulgen-stained pollen mother cells (PMCs) from* L. perenne *and each of the seven monosomic substitution lines*

		Frequency of meiotic configurations in 50 PMCs*			
Monosomic substitution line	Total no. of chiasmata*	I univalent	II bivalent	III trivalent	IV quadrivalent
1	515 (10·30)	4 (0·08)	345 (6·90)	2 (0·04)	0 (0)
2	542 (10·84)	11 (0·22)	340 (6·90)	3 (0·06)	2 (0·04)
3	512 (10·24)	16 (0·32)	342 (6·84)	0 (0)	0 (0)
4	502 (10·40)	22 (0·44)	312 (6·24)	10 (0·20)	6 (0·12)
5	478 (9·56)	33 (0·66)	332 (6·64)	1 (0·02)	0 (0)
6	498 (9·96)	16 (0·32)	340 (6·80)	0 (0)	1 (0·02)
7	496 (9·92)	54 (1·08)	323 (6·46)	0 (0)	0 (0)
L. perenne (Liprior) Ba10890/8 (recurrent crossing parent)	231 (9·24)	4 (0·16)	173 (6·92)	0 (0)	0 (0)

*The means per cell are shown in parentheses.

the results from the marker data. Thus any breakdown in synteny between *L. perenne* and *F. pratensis* that could lead to rare multivalent formation is likely to be complex, e.g. involve proximal translocations between the *F. pratensis* and *L. perenne* genomes.

Effective alien introgression strategies require high rates of homoeologous recombination between host and alien chromosomes (or very large populations and multiple generations) in order to minimize the transfer of an undesirable genetic load via linkage drag from the alien species. Recombination between homoeologous chromosomes within the forage grasses has previously been shown not to be compromised by differences in DNA content in terms of either total DNA content or repetitive sequences (Jenkins *et al.*, 2000). Indeed, high rates of recombination between host *L. perenne* and alien *F. pratensis* chromosomes have already been observed for the chromosome 3 monosomic substitution line (King *et al.*, 2007*b*). The cytogenetic observations confirm that all seven chromosomes are likely to recombine, though possibly with some limitations (the low numbers of univalents and multivalent observed). Work on a related diploid hybrid, *L. temulentun* × *L. perenne*, which also contained two sets of chromosomes which were genetically and structurally dissimilar, showed the hybrid to have a remarkable capacity to eliminate synaptonemal complex irregularities (which would interfere with pairing) and to produce homoeologous bivalents (Jenkins and White, 1990).

TABLE 3. *GISH analysis of metaphase I of 25 pollen mother cells (PMCs) of each monosomic substitution line*

Monosomic substitution line	Total no. of chiasma and mean per cell	Frequency of meiotic configurations in 25 PMCs (%)				Average chiasma frequency		No. of *Lp/Fp* rods and rings	
		I univalent	II bivalent	III trivalent	IV quadrivalent	For all bivalents, i.e. *Lp/Lp* and *Lp/Fp*	For *Lp/Fp* bivalents	Rods	Rings
1	286 11·44	1 (0) 0·04 (0)	173 (24) 6·92 (0·96)	1 (1) 0·04 (0·04)	0 0	1·64	1·33	16	8
2	308 12·32	3 (1) 0·12 (0·04)	168 (22) 6·72 (0·88)	1 (1) 0·04 (0·04)	2 (1) 0·08 (0·04)	1·77	1·86	3	19
3	293 11·72	2 (1) 0·08 (0·04)	174 (24) 6·96 (0·96)	0 0	0 0	1·67	1·84	2	22
4	283 11·32	2 (0) 0·08 (0)	167 (21) 6·68 (0·84)	2 (2) 0·08 (0·04)	2 (2) 0·08 (0·04)	1·62	1·90	2	19
5	292 11·68	2 (1) 0·08 (0·04)	174 (24) 6·96 (0·96)	0 0	0 0	1·67	1·25	18	6
6	309 12·36	0 0	175 (25) 7 (1·00)	0 0	0 0	1·76	1·80	5	20
7	275 11·00	4 (2) 0·16 (0·08)	173 (23) 6·92 (0·92)	0 0	0 0	1·57	1·43	13	10

TABLE 4. *Characteristics of some Pooideae grasses and cereals*

Species	*Brachypodium distchyon*	*Lolium perenne*	*Hordeum vulgare*	*Secale cereale*	*Avena sativa*	*Triticum aestivum*
Common name	Purple false brome	Perennial ryegrass	Barley	Rye	Oats	Wheat
Tribe	Brachypodieae	Poeae	Triticeae	Triticeae	Aveneae	Triticeae
Chromosome no. (*n*)	5	7	7	7	7	7
Ploidy	2*n*	2*n*	2*n*	2*n*	6*n*	6*n*
Approx. genome size (Gb)	0·35	2·3	5·5	8·1	13	17
Agronomic status	Uncultivated	Cultivated	domesticated	Domesticated	Domesticated	Domesticated
Breeding system	Inbreeder	Outbreeder	Inbreeder	Outbreeder	Inbreeder	Inbreeder
Crop*	–	Forage/seed	Grain	Grain/forage	Grain	Grain
Life cycle	Annual	Perennial	Annual	Annual/perennial	Annual	Annual

* Current major application(s) in the UK. Ryegrass also has many amenity uses and grain crops can be used as forages.

In this work we have described the transfer of the entire genome of *F. pratensis* into *L. perenne* through the development of seven monosomic substitution lines. This germplasm provides a unique source of genetic variation that can be exploited for the determination of the genetic control of target traits and the subsequent development of superior *L. perenne* varieties. Presently each of the seven substitutions are being screened for phenotypic variation for a range of traits that include tolerance to drought/cold, root morphology, nutrient use efficiency, disease resistance (Armstead *et al.*, 2006*b*), etc. This will allow the identification of which of the seven *F. pratensis* chromosomes carries target genes for specific traits. Once a specific *F. pratensis* chromosome has been identified as carrying a gene controlling a target trait its exact position can be determined accurately via the exploitation of *L. perenne/F. pratensis* recombinant chromosome lines, i.e. a series of lines that carry small *F. pratensis* chromosome segments derived from the target monosomic substitution (J. King *et al.*, unpubl. res.), thus providing a platform for the map-based cloning of the target gene(s) as previously demonstrated for the gene responsible for Mendel's *I* locus (Donnison *et al.*, 2005; Moore *et al.*, 2005; Armstead *et al.*, 2006*a*, 2007; Ougham *et al.*, 2008).

Within the Poaceae, the subfamily Pooideae contains ryegrasses, fescues, wheat, barley, rye oats and the model grass *Brachypodium distachyon* (purple false broom). The similarities and difference between these species (Table 4) present general opportunities for 'comparing and contrasting' across species in terms of general biology and, specifically, evolutionary and comparative genomics. For all the economically important grasses and cereals in this subfamily, alien introgression has been considered to be a useful route for introducing new traits (Humphreys *et al.*, 2003; Hodgkin and Hajjar, 2008; Nevo and Chen, 2010). Particularly in wheat, the development of comprehensive collections of monosomic substitution and deletion lines has been a major target for both cytogeneticists and geneticists and, in parallel, wheat plant breeders have developed many alien introgression approaches for improving wheat germplasm. To these ends, a wide variety of wheat wild and cultivated relatives have been used in alien introgression programmes, e.g. rye, *Triticum* spp. *Aegilops* spp, *Thinopyron* spp. (Hohmann *et al.*, 1996; King *et al.*, 1997; Friebe *et al.*, 1999; Ehdaie *et al.*, 2003; Gupta *et al.*, 2005; Jauhar *et al.*, 2009; Motsnyi *et al.*, 2009). In rye, barley and oats, while the range of species targeted for introgression is not so wide, considerable effort has still been expended in these directions (Rooney *et al.*, 1994; Prieto *et al.*, 2001; von Korff *et al.*, 2004; Lukaszewski, 2006; Atienza *et al.*, 2007; Schmalenbach *et al.*, 2008, 2009; Falke *et al.*, 2009; Fetch *et al.*, 2009; Johnston *et al.*, 2009; Schmalenbach and Pillen, 2009; Scholz *et al.*, 2009). In contrast to the Pooideae crop species, *B. distachyon* is being developed as a model monocot with the understanding that its biology will be

more analogous with the important cereals and grasses than that of *Arabidopsis thaliana*. However, within the genus *Brachypodium* there is also a wide variety of alien resources (Garvin *et al.*, 2008; Ozdemir *et al.*, 2008; Bakker *et al.*, 2009) and alien introgression is likely to prove increasingly informative as a way of extending the (otherwise narrow) range of variation and thus increasing our capacity to understand and extrapolate from the biology of this model species, just has been the case with *A. thaliana* and its relatives (Ramos-Onsins *et al.*, 2004; de Meaux *et al.*, 2006; Frerot *et al.*, 2010). The continuing development of next-generation sequencing (NGS) technologies has meant that the 'art of the possible' in genome analysis has changed to such an extent that detailed genomic characterizations of all these species are imminent (Varshney *et al.*, 2009; Deschamps and Campbell, 2010). The implication of this is that the ability to develop DNA-based cross-references between these similar but also contrasting Pooideae genomes will become routine within the next few years. Genome-wide alien introgression is a tool for generating variation which is tractable to NGS-related analyses and, thus, these analyses provide the means for cataloguing the available variation at the level of molecular genomics. There remains the major challenge in associating genomic introgression with phenotype, though the development of dedicated, large-scale phenomics facilities might mitigate this. In addition, the implementation of parallel analyses at the levels of genotype and phenotype in the Pooideae species described in Table 4 should also help to leverage extra information and suggest further avenues of investigation. Hence the genome-wide alien introgression resource for *L. perenne* and *F. pratensis* described here will not only contribute to our knowledge of the biology of the ryegrasses and fescues and to our ability to manipulate their genomes for food security, but will also contribute to the generation of a model for understanding and exploiting the variation across the Pooideae genomes.

The germplasm developed is freely available upon request to the corresponding author.

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