RESEARCH

Transmission Frequencies of Introgressed *Festuca pratensis* Chromosomes and Chromosome Segments in *Lolium perenne*

Julie King,* Ian Armstead, John Harper, and Ian King

ABSTRACT

The introgression of genetic variation from related species into crops provides an important route by which superior plant varieties can be produced. The primary aim of introgression involves the transfer of a small chromosome segment from a related species into a chromosome of a crop species (via recombination at meiosis) to generate an interspecific recombinant chromosome. Very little is known about the selective pressures that act on the products of interspecific recombination. Seven monosomic substitution lines were developed between *Lolium perenne* and *Festuca pratensis*. When each line was backcrossed to *L. perenne* recombination occurred between the *F. pratensis* chromosome and its *L. perenne* homoeologue, resulting in backcross populations carrying *L. perenne/F. pratensis* recombinant chromosomes. This paper describes the relationship between the frequency of generation of interspecific recombinant chromosomes with the frequency of their transmission to the next generation. The results reveal the presence of neutral, negative, and positive selection pressures for the transmission of *F. pratensis* chromosomes and *L. perenne/F. pratensis* recombinant chromosomes through the gametes to the next generation. The type of selection pressure observed depended on which linkage group the *F. pratensis* chromosome under study was derived from. The implications of these results are discussed.

J. King, and I. King, Ancestral Introgression Group, School of Biosciences, The Univ. of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire LE12 5RD United Kingdom; I. Armstead, and J. Harper IBERS, Gogerddan, Aberystwyth Univ., SY23 3EE, Wales, United Kingdom. Received 31 Mar. 2013. *Corresponding author (j.king@nottingham.ac.uk).

Abbreviations: GISH, genomic in situ hybridization; SSR, Simple Sequence Repeats.

The role of interspecific hybridization in plant evolution is well known (Stebbins, 1950), i.e., many of the world's cultivated crops are allopolyploid in origin, e.g., *Triticum aestivum* (wheat), *Avena sativa* (oats), *Gossypium hirsutum* (cotton), *Nicotiana tabacum* (tobacco). Interspecific hybrids also provide the starting point for the introgression of genes from one species into another. In crop species the introgression of agronomically important genes from related species provides a valuable source of genetic variation which can be exploited for the development of superior, high yielding, adapted plant varieties (King et al., 2013).

Introgression, in its simplest form, involves the sexual hybridization of different species to form an inter-specific F_1 hybrid. Ancestral introgression occurs in the F_1 hybrid (or its derivatives) when related, homoeologous chromosomes from the two parental species (i.e., chromosomes that carry orthologous genes in essentially the same order) recombine at meiosis resulting in the generation of interspecific recombinant chromosomes. These recombinant chromosomes are then transmitted to the next generation through the gametes. The repeated backcrossing of the F_1 hybrid to one of the parental genotypes results in the generation of lines which carry the majority of the genome of one species but also

Published in Crop Sci. 53:1968–1973 (2013).
doi: 10.2135/cropsci2013.03.0209

carry one or more introgressed chromosome segments from the other parental species.

The genus *Lolium* is composed of a number of species all of which are diploid ($2n = 2x = 14$) with a basic chromosome number of seven. In contrast the genus *Festuca*, again with a basic number of seven, is more diverse including both diploid and polyploid species, i.e., diploid to decaploid (Thomas and Humphreys, 1991). Many of the *Lolium* and *Festuca* species can form intergeneric hybrids and some of the resulting hybrids are fertile, e.g., *L. perenne/L.perenne/F. pratensis* triploid hybrids show significant levels of fertility (King et al., 1998, 1999, 2002a, 2002b, 2008, 2013). The four major agricultural species within the *Lolium-Festuca* complex, *L. multiflorum* (Italian ryegrass), *L. perenne* (perennial ryegrass), *F. pratensis* (meadow fescue), and *F. arundinacea* (tall fescue), are the most important sources of forage grass in temperate regions of the world. The *Lolium* species provide the highest quality forage in terms of digestibility with good soluble carbohydrate content while the *Festuca* species are adapted to more extreme winter cold and summer drought. The combination of cold and heat tolerance from the *Festuca* species, via introgression, to *Lolium* would have significant agricultural benefits.

To facilitate the introgression of genes from *F. pratensis* ($2n = 2x = 14$) into *L. perenne*, seven monosomic substitution lines were developed. Each of the seven lines has a different *L. perenne* chromosome (from a different genetic linkage group) replaced by a homoeologous chromosome from *F. pratensis* (Harper et al., 2011). Each of these monosomic substitution lines has been backcrossed to *L. perenne*. Recombination between the *F. pratensis* chromosome and the *L. perenne* homoeologue in each monosomic substitution line occurs freely (Harper et al., 2011). Thus, the backcross family progeny derived from each of the seven monosomic substitution lines carried *L. perenne/F. pratensis* recombinant chromosomes. This research programme resulted in the introgression of the entire genome of *F. pratensis* into the *L. perenne* genome in overlapping chromosome segments (King et al., 2013). The germplasm developed is providing an important resource for: (ii) variety development, (ii) comparative mapping and evolutionary studies, (iii) the physical mapping and sequencing of the forage grass genome, and (iv) recombination studies.

Interspecific recombinant chromosomes normally show stable inheritance once they become homozygous, e.g., the lines of *L. multiflorum* which carry a gene for delayed senescence (Armstead et al., 2006, 2007; Moore et al., 2005), lines of wheat which carry a 1BL. 1RS translocation (Ammar et al., 2004). The stable transmission results from normal meiotic behavior, i.e., pairing and synapsis, etc., that occurs due to the homozygosity of these interspecific recombinant chromosomes.

However, very little is known about the selective pressures that act on the transmission of the products of interspecific recombination directly after their formation before their appearance in the next generation. In the work of King et al. (2007, 2013) sites of recombination between the *L. perenne* and *F. pratensis* chromosomes in each of the seven monosomic substitution lines were recovered in the backcross progenies generated. However, the work reported did not address whether selection pressures acted on the transmission of interspecific recombinant chromosomes and nonrecombinant chromosomes generated at meiosis in each of the monosomic substitution lines. To do this it is first necessary to study the frequency of recombination between the homoeologous *L. perenne/F. pratensis* bivalents in each of the seven substitution lines.

This paper examines the transmission of interspecific recombinant chromosomes generated at meiosis in each of the seven *L. perenne/F. pratensis* substitution lines. Specifically, we (i) determine the frequency at which interspecific recombinant and nonrecombinant chromosomes are generated, during meiosis and gamete formation, in each of seven different *L. perenne/F. pratensis* substitution lines using data from genomic in situ hybridization (GISH) analysis (King et al., 2002b; Harper et al., 2011), and (ii) relate this information to the actual frequencies at which interspecific recombinant and nonrecombinant chromosomes are transmitted to the next generation through the male gametes.

MATERIALS AND METHODS

The germplasm being used to undertake this study are seven *L. perenne/F. pratensis* monosomic substitution lines ($2n = 2x = 14$, i.e., 13 *L. perenne* chromosomes + 1 *F. pratensis* chromosome). These seven lines each have a single *L. perenne* chromosome, from a different linkage group, replaced by a homoeologous chromosome from *F. pratensis*, (Harper et al., 2011). The lines were developed by crossing a synthetic tetraploid *L. perenne* 'Meltra' (female parent) with diploid *F. pratensis* (male parent) to create an interspecific F_1 triploid *L. perenne/L. perenne/F. pratensis* hybrid. The interspecific triploid hybrid was then backcrossed as the male parent to *L. perenne* ('Liprio') to produce a BC_1 population from which the seven monosomic substitution lines were isolated.

Each of the seven substitution lines was then backcrossed again as the male parent to the same diploid *L. perenne* Liprio genotype used previously (King et al., 2007, 2013) to produce seven BC_2 mapping populations. The BC_2 populations were genetically mapped using Simple Sequence Repeats (SSRs) generated by Vialactia Biosciences (Gill et al., 2006), the Institute of Grassland and Environmental Research (IGER) (King et al., 2008) and those available in the public domain, which were polymorphic for *F. pratensis* relative to *L. perenne* and which are distributed throughout the seven linkage groups of *L. perenne/F. pratensis* (Table 1).

Classification of individuals was as follows: individuals making up each mapping population were screened with all the SSRs from the relevant linkage group. Those individuals showing polymorphism for all the SSRs were classified as carrying a complete *F. pratensis* chromosome. Individuals lacking all of the polymorphisms from the relevant linkage group were classified as lacking the *F. pratensis* chromosome and carrying two

Table 1. Microsatellite markers used to characterize the progeny derived from each of the 7 monosomic substitution lines.

Subline 1	Subline 2	Subline 3	Subline 4	Subline 5	Subline 6	Subline 7
RV1391†	RV0226	RV0774	RV0454	RV1200	RV0144	RV0009
NFA134‡	NFA023	LpACA8F7	NFA071	RV1112	RV1266	RV1411
NFA073	RV1008	RV1046	RV1087	RV1103	Rye014	RV0011
RV0137	RV1269	RV0680	LpSSRH01H06	RV0950	RV1093	RV1171
LpACT15H3§	RV0062	NFA099	RV0966	RV0809	RV0970	08ga1
B1B6¶	RV1068	B3B8	RV0922	RV1139	RV0830	LpACA11D4
17ga1†	RV1268	RV1131	NFA104	B2F1	RV0297	RV0333
RV0033	NFA092	RV0113	LpACA8B9	RV0184	RV1036	DLF008
RV0301	RV1396	RV1390	RV0650	RV0157	RV0609	LpACT13F9
RV0003	LpSSRH01A07††	RV0756	LpHCA21H1	LpACT1B5	RV0067	RV1254
B3B1	RV1031	B1C9	RV1295	RV0342	RV0985	RV0620
PR8#	RV1239	RV1172	RV0785	RV0054	NFA036	RV0264
PR25	RV1164	B5F9	RV0668	RV1307	NFA048	B3C5
RV0624		RV1439	RV1127	NFA059	NFA111	LpACT26G3
RV0394		RV0753	RV1056	RV0250	LpACT14C9	RV0817
LpSSR085#		LpHCA21E6a	RV0380	RV0752	LpACT26G8	RV1408
LpACA11H9		RV0863	NFA142	LpACA11G10b	LpACA24B4	
NFA088			RV0551		RV0641	
PR37			RV0810		RV1365	
RV0367			RV0161		RV1208	
RV0165			RV1190			
NFA140			RV0372			
LpACA30G7b			RV1053			
			RV1153			

†Vialactia Biosciences (Gill et al., 2006).

‡Saha et al. (2004).

§King et al. (2008).

¶Lauvergeat et al. (2005).

#Jensen et al. (2005).

††Jones et al. (2001).

complete *L. perenne* chromosomes for that linkage group, i.e., a complete *L. perenne* chromosome had been transmitted to the progeny from the substitution line. Individuals carrying some, but not all, *F. pratensis* specific SSR polymorphisms from a linkage group were classified as carrying an interspecific *L. perenne/F. pratensis* recombinant chromosome. To confirm the efficacy of this approach, GISH was used to screen a selection of individuals to confirm the presence or absence of recombinant chromosomes (King et al., 2013). The data obtained from this analysis represents the ***observed*** transmission frequencies of interspecific recombinants and nonrecombinants through the pollen.

It is possible to predict the type and frequency of recombinant and nonrecombinant chromatids (and hence the number of exchanges) generated, when either one or two chiasmata are formed within the *L. perenne/F. pratensis* bivalents in each of the seven substitution lines at meiosis assuming that the ratio of rods (one chiasmate bivalents) to rings (two chiasmate bivalents with at least one chiasma in each arm) is known and that there is no chromatid interference (Kearsey and Pooni, 1996) (Note the absence of chromatid interference in *L. perenne/F. pratensis* monosomic substitution lines was demonstrated by King et al., 2002b). Bivalents involving two chiasmata give rise to types and proportions of recombinant chromatids that differ to those involving one chiasma. Bivalents with one chiasma will always give rise to 50% recombinant and 50% nonrecombinant chromosomes (Kearsey and Pooni, 1996). In the absence of chromatid interference, the position of the second chiasma in bivalents with two chiasmata is expected to be independent of the first in terms of the chromatids involved. Thus 50% of two chiasmate bivalents will involve three strand exchanges while the remaining 50% will be composed of equal numbers of two and four strand exchanges. Overall two chiasmate bivalents will therefore be expected to give rise to 25% nonrecombinants, 50% single recombinants and 25% double recombinants (Kearsey and Pooni, 1996).

The frequency of rod and ring formation between the *F. pratensis* chromosomes and their *L. perenne* homoeologues has previously been determined via GISH (Harper et al., 2011) (Table 2). This data therefore enables the ***expected*** frequency of the generation of interspecific recombinant to nonrecombinant chromosomes, during gamete formation in each of the seven monosomic substitution lines, to be determined as described above.

RESULTS

One thousand four hundred and six progeny were generated from crosses between the seven substitution lines (pollen parents) and *L. perenne* (female parent). The total number of progeny derived from each substitution line is shown in Table 3. These progeny were screened with 130 SSRs (Table 1). The observed frequencies of transmission of complete *L. perenne and F. pratensis* chromosomes and *L. perenne/F. pratensis* recombinant chromosomes are shown in Table 3. The expected frequency of transmission of nonrecombinant and *L. perenne/F. pratensis* recombinant chromosomes is also shown in Table 3.

Table 2. Frequency of rod and ring formation between the *L. perenne* and homoeologous *F. pratensis* chromosome in each monosomic substitution line (data modified from Harper et al., 2011).

Monosomic substitution line	Frequency of *L. perenne/F. pratensis* rod bivalents	Frequency of *L. perenne/F. pratensis* ring bivalents
	(%)	
1	66.67	33.33
2	13.64	86.36
3	8.33	91.67
4	9.52	90.48
5	75	25
6	20	80
7	57	43

Observed versus expected frequencies of nonrecombinant *L. perenne* and *F. pratensis* chromosomes and *L. perenne/F. pratensis* recombinant chromosomes were compared using χ^2 analyses (Table 3). The progenies derived from substitution lines 1, 2, 4, 5, and 6 show significant deviations between the expected and observed frequencies of nonrecombined *L. perenne* and *F. pratensis* and recombinant *L. perenne/F. pratensis* chromosomes. In the progenies derived from substitution lines 2, 4, 5, and 6 far fewer *L. perenne/F. pratensis* recombinant chromosomes were observed than expected, i.e., far more nonrecombined chromosome were observed than expected. The greatest deviation was found in the progenies derived from substitution lines 4 and 6 where the transmission of *L. perenne/F. pratensis* chromosomes to the next generation was very low.

In the absence of any form of selection, the transmission of nonrecombined *L. perenne* and *F. pratensis* chromosomes to the next generation will occur at equal frequency, i.e., a 1:1 ratio. However, significantly fewer nonrecombined *F. pratensis* chromosomes were observed in the progenies derived from substitution lines 2, 4, 5, 6, and 7 (Table 4). Here again the greatest deviation was observed in the progenies derived from substitution lines 4 and 6. A complete nonrecombined *F. pratensis* chromosome was not observed in either of these progenies even though they were the largest observed, i.e., 345 and 315 individuals, respectively.

The progeny derived from substitution line 1 also showed a significant deviation between the observed and expected frequencies of nonrecombined *L. perenne* and *F. pratensis* chromosomes and *L. perenne/F. pratensis* recombinant chromosomes (Table 3). However, in contrast to the progenies derived from substitution lines 2, 4, 5, and 6, here the frequencies of *L. perenne/F. pratensis* recombinant chromosomes was far higher than expected with only one individual in a population of 73 carrying a nonrecombined chromosome.

The progeny derived from substitution lines 3 and 7 were the only ones which did not show a significant difference between the observed and expected numbers of recombinant and nonrecombinant *L. perenne/F. pratensis* chromosomes. However, as with the progenies from substitution lines 2, 4, 5, and 6, the number of nonrecombined *F. pratensis* chromosomes observed in the progeny derived from substitution line 7 was significantly less than the number of nonrecombined *L. perenne* chromosomes (Table 4).

DISCUSSION

In this work, the expected frequencies of *L. perenne/F. pratensis* recombinant chromosomes and nonrecombinant *L. perenne* and *F. pratensis* were calculated based on chiasma frequency in each of the seven substitution lines (Harper et al., 2011). Chiasmata are the major source of recombination in organisms (Tease and Jones, 1978; King et al., 2002b). Even though other phenomena such as gene conversion (Jeffreys et al., 1994; Broman et al., 1998), and arguably chiasma terminalization (Darlington, 1929; Tease and Jones, 1995), also lead to recombinant events their frequency is sufficiently low that it is very difficult to detect in mapping populations. This results in a 1:1 correspondence between recombination frequency, i.e., genetic distance and chiasma frequency (Hultén, 1974; Tease and Jones, 1978, 1995; King et al., 2002b). Thus, providing that the key parameters are known, it is possible to predict the products of meiosis of an individual bivalent (Kearsey and Pooni, 1996; King et al., 2002b).

In the work described here. the chiasma frequencies in each of the *L. perenne/F. pratensis* bivalents are known and with very few exceptions only one chiasma is formed in each chromosome arm (Harper et al., 2011; King et al., 2002b, 2007, 2013). In addition, chromatid interference across the centromere does not occur in *L. perenne/F. pratensis* homoeologous bivalents (King et al., 2002b). This information therefore enabled the frequency of *L. perenne/F. pratensis* recombinants and nonrecombinants to be estimated as previously described by King et al. (2002b).

The analysis undertaken revealed negative (progenies derived from monosomic substitution lines 2, 4, 5, and 6), positive (progeny derived from monosomic substitution line 1), and neutral (progenies derived from monosomic substitution lines 3 and 7) selection for the transmission of *L. perenne/F. pratensis* recombinant chromosomes through the gametes of the seven monosomic substitution lines.

The transfer of genetic variation from the distant relatives of our key crop species, i.e., ancestral introgression, provides an important and largely untapped source of genetic variation for agronomically important traits that can be utilized by plant breeders for the development of superior, high yielding, adapted crops. In the monocots, ancestral introgression has already had a significant impact on agricultural production. Examples in the grasses include genes that confer resistance to disease and tolerance to abiotic stress, etc. (e.g., Roderick et al., 2003; Armstead et al., 2006; Humphreys et al., 2005, 2007). In wheat, examples include introgressions from *Aegilops umbellulata,* e.g., leaf rust resistance (which saved U.S. wheat production from catastrophic failure in 1960; Sears, 1956, 1972); resistance

Table 3. Chi-square analyses of observed versus expected frequency of nonrecombined *L. perenne* and *F. pratensis* chromosomes and recombined *L. perenne/F. pratensis* chromosomes.†

Substitution line‡	Nonrecombined *L.perenne* and *F. pratensis* chromosomes		Recombined *L.perenne/ F. pratensis* chromosomes		χ^2	Significance¶
	Observed§	Expected	Observed	Expected		
1 (73)	1 (1*Lp*, 0*Fp*)	30	72	43	47.59	***
2 (207)	89 (75*Lp*, 14*Fp*)	59	118	148	21.33	***
3 (114)	40 (25*Lp*, 15*Fp*)	31	74	83	3.6	NS
4 (345)	307 (307*Lp*, 0*Fp*)	95	38	251	653.85	***
5 (186)	138 (87*Lp*, 51*Fp*)	81	48	105	71.05	***
6 (315)	253 (253*Lp*, 0*Fp*)	95	62	220	376.25	***
7 (166)	69 (50*Lp*, 19*Fp*)	65	97	101	0.41	NS

***Significant at the 0.001 probability level.

†An example (from King et al., 2002b) of expected numbers calculation of *L. perenne/F. pratensis* recombinants and *L. perenne* and *F. pratensis* nonrecombinants using the data from monosomic substitution line 1 as an example is as follows:

1. Rod: rings, 66.7%: 33.33% (Table 2).
2. Rods give rise to 50% nonrecombinants and 50% recombinants.

Thus if 66.7% of bivalents observed were rods then 50% (66.7% ÷ 2), i.e. 33.33% would be nonrecombinants and 50% (66.7% ÷ 2), i.e. 33.33% would be recombinants.

3. Rings = 25% nonrecombinants, 50% single recombinants and 25% double recombinants. Thus if 33.33% of bivalents observed were rings then 25% (33.33 ÷ 4), i.e. 8.33%, would be nonrecombinants, 50% (33.33 ÷ 2), i.e. 16.67%, would be single recombinants and 25% (33.33 ÷ 4), i.e. 8.33%, would be double recombinants.
4. The total number of nonrecombinants that would be expected in the progeny of monosomic substitution line 1 is therefore 33.33% (rods) + 8.33%(rings) = 41.66%. Since the total number of progeny derived from monosomic substitution line 1 was 73, 30 of these (41.66% of 73) would be expected to be nonrecombinants.
5. The total number of *L. perenne/F. pratensis* interspecific recombinants that would be expected 33.335% (rods) + 16.67% (rings) + 8.33% (rings) = 58.33%. Thus out of a population of 73, 43 (58.33% of 73) would be expected to be recombinants.

‡Numbers in parentheses represent total number of individuals screened with SSRs from each of the seven mapping populations (derived from crossing each substitution line with *L. perenne*).

§Numbers in parentheses represent number of nonrecombined *L. perenne* and *F. pratensis* chromosomes observed in each of the seven mapping populations (derived from crossing each substitution line with *L. perenne*).

¶For 1 degree of freedom the 0.05 level of significance is 3.84 and the 0.001 level of significance is 10.83.

Table 4. Chi-square analyses of observed frequency of nonrecombined *L. perenne* and *F. pratensis* chromosomes.

Substitution line	Number of nonrecombined *L. perenne* chromosomes‡	Number of nonrecombined *F. pratensis* chromosomes§	Total number of nonrecombined chromosomes	χ^2	Significance
1†	1	0	1	–	–
2	75 (44.5)	14 (44.5)	89	41.8	***
3	25 (20)	15 (20)	40	2.5	NS
4	307 (153.3)	0 (153.3)	307	307	***
5	87 (69)	51 (69)	138	9.4	*
6	253 (126.5)	0 (126.5)	253	253	***
7	50 (34.5)	19 (34.5)	69	69	***

*Significant at the 0.05 probability level.

***Significant at the 0.001 probability level.

†It was not possible to perform an analysis on the nonrecombinant chromosomes in the progeny derived from monosomic substitution line 1 as only one individual carried a nonrecombined chromosome.

‡The expected numbers of nonrecombined *L. perenne* chromosomes are shown in brackets.

§The expected numbers of nonrecombined *F. pratensis* chromosomes are shown in brackets.

to a range of diseases, tolerance to acid soils, increased yield advantage and stability from rye (Ammar et al., 2004; McIntosh, 1983); a gene from *Ae. ventricosa* conferring resistance to eyespot (Doussinault et al., 1983); many of the top wheat varieties in Europe, e.g., "Robigus", are derived from unknown introgressions from *Triticum dicoccoides*.

The transmission of chromosomes and/or recombinant chromosomes from ancestral species through the gametes of interspecific hybrids and their derivatives, as demonstrated in this work, is of significant importance since the selective pressures operating will impact on the ease of introgressing genetic material from ancestral species into crops, e.g., it will be very difficult to transfer a gene from a related species into a crop if low levels of recombination occur between homoeologous chromosomes and significant selection acts against the introgressed chromosome and subsequent homoeologous recombinants.

From an evolutionary standpoint, it is interesting to consider the consequences of the generation of interspecific hybrids where there is considerable positive selection for a homoeologous recombinant chromosome(s) as shown with the progeny derived from monosomic substitution line 1, i.e., if an interspecific hybrid and its progeny underwent multiple rounds of backcrossing to one parent would the new interspecific recombinant chromosome spread throughout the population?

Acknowledgments

The authors would like to thank the BBSRC for funding this research.

References

Ammar, K., M. Mergoum, and S. Rajoram. 2004. The history and evolution of triticale. In: M. Megoum and H. Gomez-Macpherson, editors, Triticale improvement and production. FAO Plant Prodcution and Protection series No. 179. FAO Rome, Italy. p. 1–11.

Armstead, I.P., I.S. Donnison, S. Aubry, J.A. Harper, S. Hortensteiner, C.L. James, et al. 2006. From crop to model to crop: Identifying the genetic basis of the staygreen mutation in the Lolium/Festuca forage and amenity grasses. New Phytol. 172:592–597. doi:10.1111/j.1469-8137.2006.01922.x

Armstead, I.P., I.S. Donnison, S. Aubry, J.A. Harper, S. Hortensteiner, C.L. James, et al. 2007. Cross-species identification of Mendel's *I* locus. Science 315:73. doi:10.1126/science.1132912

Broman, K.W., J.C. Murray, V.C. Sheffield, R.L. White, and J.L. Weber. 1998. Comprehensive human genetic maps: Individual and sex specific variation in recombination. Am. J. Hum. Genet. 63:861–869. doi:10.1086/302011

Darlington, C.D. 1929. Chromosome behavior and structural hybridity in the Tradescantiae. J. Genet. 21:207–286. doi:10.1007/BF02984208

Doussinault, G., A. Delibes, R. Sanchez-Monge,and F. Garcia-Olmedo. 1983. Transfer of a dominant gene for resistance to eyespot disease from a wild grass to hexaploid wheat. Nature 303: 698–700.

Gill, G.P., P.L. Wilcox, D.J. Whittaker, R.A. Winz, P. Bickerstaff, C.E. Echt, et al. 2006. A framework linkage map of perennial ryegrass based on SSR markers. Genome 49:354–364. doi:10.1139/G05-120

Harper, J.A., I.P. Armstead, A. Thomas, C. James, D. Gasior, M. Bisaga, et al. 2011. 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. Ann. Bot. (Lond.) 107:1313–1321. doi:10.1093/aob/mcr083

Hultén, M. 1974. Chiasma distribution at diakinesis in the normal human male. Hereditas 76:55–78. doi:10.1111/j.1601-5223.1974.tb01177.x

Humphreys, J., J.A. Harper, I.P. Armstead, and M.W. Humphreys. 2005. Introgression mapping of genes for drought resistance transferred from *Festuca arundinacea* var. glaucescens into *Lolium multiflorum*. Theor. Appl. Genet. 110:579–587. doi:10.1007/s00122-004-1879-2

Humphreys, M.W., D. Gasior, A. Lesniewska-Bocianowska, Z. Zwierzykowski, and M. Rapacz. 2007. Androgenesis as a means of dissecting complex genetic and physiological controls: Selecting useful gene combinations for breeding freezing tolerant grasses. Euphytica 158:337–345. doi:10.1007/s10681-006-9240-2

Jeffreys, A.J., K. Tamaki, A. MaCleod, D.G. Monckton, D.L. Neil, and J.A.L. Armour. 1994. Complex gene conversion events in germline mutation at human minisatellites. Nat. Genet. 6:136–145. doi:10.1038/ng0294-136

Jensen, L.B., H. Muylie, P. Arens, C.H. Andersen, P.B. Holm, M. Ghesquiere, et al. 2005. Development and mapping of a public reference set of SSR markers in *Lolium perenne* L. Mol. Ecol. Notes 5:951–957. doi:10.1111/j.1471-8286.2005.01043.x

Jones, E.S., M.P. Dupal, R. Kolliker, M.C. Drayton, and J.W. Forster. 2001. Development and characterization of simple sequence repeat (SSR) markers for perennial ryegrass (*Lolium perenne* L.). Theor. Appl. Genet. 102:405–415. doi:10.1007/s001220051661

Kearsey, M.J., and S. Pooni. 1996. The genetical analysis of quantitative traits. Chapman and Hall, London. p. 116–119.

King, I.P., W.G. Morgan, I.P. Armstead, J.A. Harper, M.D. Hayward, A. Bollard, et al. 1998. Introgression mapping in the grasses I. Introgression of *Festuca pratensis* chromosomes and chromosome segments into *Lolium perenne*. Heredity 81:462–467. doi:10.1046/j.1365-2540.1998.00437.x

King, I.P., W.G. Morgan, J.A. Harper, and H.M. Thomas. 1999. Introgression mapping in the grasses. II. Meiotic analysis of the *Lolium perenne/Festuca pratensis* triploid hybrid. Heredity 82:107–112. doi:10.1038/sj.hdy.6884680

King, J., I.P. Armstead, I.S. Donnison, H.M. Thomas, R.N. Jones, M.J. Kearsey, et al. 2002a. Physical and genetic mapping in the grasses *Lolium perenne* and *Festuca pratensis*. Genetics 161:315–324.

King, J., I.P. Armstead, S.I. Donnison, L.A. Roberts, J.A. Harper, K. Skøt, et al. 2007. Comparative analyses between Lolium/Festuca introgression lines and rice reveal the major fraction of functional annotated gene models are located in recombination poor/very poor regions of the genome. Genetics 177:597–606. doi:10.1534/genetics.107.075515

King, J., I. Armstead, J. Harper, L. Ramsey, J. Snape, R. Waugh, et al. 2013. Exploitation of interspecific diversity for monocot crop improvement. Heredity 110:475–483. 10.1038/hdy.2012.116.

King, J., L.A. Roberts, M.J. Kearsey, H.M. Thomas, R.N. Jones, L. Huang, et al. 2002b. A demonstration of a 1:1 correspondence between chiasma frequency and recombination using a *Lolium perenne/Festuca pratensis* substitution. Genetics 161:307–314.

King, J., D. Thorogood, K.J. Edwards, I.P. Armstead, L.A. Roberts, K. Skøt, et al. 2008. Development of a genomic microsatellite library in perennial ryegrass (*Lolium perenne*) and its use in trait mapping. Ann. Bot. (Lond.) 101:845–853. doi:10.1093/aob/mcn016

Lauvergeat, V., P. Barre, M. Bonnet, and M. Ghesquiere. 2005. Sixty simple sequence repeat markers for use in the Festuca-Lolium complex of grasses. Mol. Ecol. Notes 5:401–405. doi:10.1111/j.1471-8286.2005.00941.x

McIntosh, R.A. 1983. A catalogue of gene symbols for wheat. In: S. Sakamoto, editor, Proceedings of the 6th International Wheat Genetics Symposium, Kyoto Japan. 28 Nov.–3 Dec. Plant Germ-Plasm Inst., Univ. of Kyoto, Japan. p. 1197–1255.

Moore, B.J., S.I. Donnison, J.A. Harper, I.P. Armstead, J. King, H. Thomas, et al. 2005. Molecular tagging of a senescence gene by introgression mapping of a mutant stay-green locus from *Festuca pratensis*. New Phytol. 165:801–806. doi:10.1111/j.1469-8137.2004.01269.x

Roderick, H.W., W.G. Morgan, J.A. Harper, and H.M. Thomas. 2003. Introgression of crown rust (*Puccinia coronata*) resistance from meadow fescue (*Festuca pratensis*) into Italian rye grass (*Lolium multiflorum*) and physical mapping of the locus. Heredity 91:396–400. doi:10.1038/sj.hdy.6800344

Saha, M.C., M.A.R. Mian, I. Eujayl, J.C. Zwonitzer, L.J. Wang, and G.D. May. 2004. Tall fescue EST-SSR markers with transferability across several grass species. Theor. Appl. Genet. 109:783–791. doi:10.1007/s00122-004-1681-1

Sears, E.R. 1956. The transfer of leaf rust resistance from *Aegilops umbellulata* to wheat. Brookhaven Symp. Biol. 9:1–22.

Sears, E.R. 1972. Chromosome engineering in wheat. Stadler Symposium 4: 23–38.

Stebbins, G.L. 1950. Variation and evolution in plants. Columbia Univ. Press, New York.

Tease, C., and G.H. Jones. 1978. Analysis of exchanges in differentially stained meiotic chromosomes of *Locusta migratoria* after Brd-U substitution and FPG staining I. Crossover exchanges in monochiasmate bivalents. Chromosoma 69:163–178. doi:10.1007/BF00329915

Tease, C., and G.H. Jones. 1995. Do chiasma disappear? An examination of whether closely spaced chiasmata are liable to reduction or loss. Chromosome Res. 3:162–168. doi:10.1007/BF00710709

Thomas, H., and M.O. Humphreys. 1991. Progress and potential of interspecific hybrids of *Lolium* and *Festuca*. J. Agric. Sci. 117:1–8. doi:10.1017/S0021859600078916