Introgression Mapping in The Grasses

I. P. King
Institute of Grassland and Environmental Research, UK

J. King
Institute of Grassland and Environmental Research, UK

I. P. Armstead
Institute of Grassland and Environmental Research, UK

J. A. Harper
Institute of Grassland and Environmental Research, UK

L. A. Roberts
Institute of Grassland and Environmental Research, UK

See next page for additional authors

The XX International Grassland Congress took place in Ireland and the UK in June-July 2005. The main congress took place in Dublin from 26 June to 1 July and was followed by post congress satellite workshops in Aberystwyth, Belfast, Cork, Glasgow and Oxford. The meeting was hosted by the Irish Grassland Association and the British Grassland Society.

Proceedings Editor: D. A. McGilloway

Publisher: Wageningen Academic Publishers, The Netherlands

Presenter Information

I. P. King, J. King, I. P. Armstead, J. A. Harper, L. A. Roberts, H. Thomas, H. J. Ougham, R. N. Jones, A. Thomas, B. J. Moore, L. Huang, and I. S. Donnison

Introgression mapping in the grasses

I.P. King[1], J. King[1], I.P. Armstead[1], J.A. Harper[1], L.A. Roberts, H. Thomas[1], H.J. Ougham, R.N. Jones[2], A. Thomas[1], B.J. Moore[1,2], L. Huang[1] and I.S. Donnison[1]
[1]*Molecular and Applied Genetics Team, Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23 3EB, Wales, United Kingdom,*
e-mail: ian.king@bbsrc.ac.uk
[2]*Institute of Biological Sciences, The University of Wales Aberystwyth, Ceredigion SY23 3DA, Wales, UK*

Key points

1. *Lolium perenne/Festuca pratensis* hybrids and their derivatives provide an ideal system for intergeneric introgression.
2. The *Lolium perenne/Festuca pratensis* system is being exploited to elucidate genome organisation in the grasses, determination of the genetic control of target traits and the isolation of markers for MAS in breeding programmes.
3. The potential of the system as an aid to contig the *Lolium* and *Festuca* genomes and for gene isolation is discussed.

Keywords: introgression mapping, introgression landing, *Lolium perenne/Festuca pratensis*, contig, gene isolation

Introduction

The *Lolium perenne* (*Lp*) and *Festuca pratensis* (*Fp*) hybrids and their derivatives exhibit a unique combination of characteristics, not seen in other plant species, which makes the *Lp/Fp* system an ideal model for intergeneric introgression.
The combination of characters exhibited by *Lp/Fp* hybrids and their derivatives include:
1. A high frequency of recombination between *Lp* and *Fp* chromosomes facilitates the transfer of *F. pratensis* chromosome segments, carrying target genes, into *Lolium.*
2. Although the chromosomes of the two species recombine at high frequency at meiosis, they can be distinguished easily through genomic *in situ* hybridisation (GISH). GISH analysis allows the identification and classification of *Lolium /Fp* introgressions, i.e. confirmation of the introgression of *Fp* segments into *Lolium*, and an estimation of their physical size.
3. Recombination occurs along the entire length of *Lolium/Fp* bivalents permitting the transfer of any *Fp* gene into *Lolium*.
4. A high frequency of marker polymorphism is observed between *Fp* and *Lolium* which aids the mapping of target *Fp* genes on introgressed *Fp* segments.
5. The system also facilitates the rapid identification of markers located on an introgressed *F. pratensis* chromosome segment by the screening of a *Lolium/Fp* introgression together with the parental and hybrid germplasm from which it was derived. Any polymorphic marker present in the *Fp* parent, the *Lolium/Fp* hybrid and the introgression line itself, but not the *Lolium* parents, must be located within the introgressed *Fp* chromosome segment.

The work described in this paper enables the elucidation of gene organization along *Lolium/Fp* chromosomes; the determination of the relationship between gene distribution and recombination, allowing comparisons to be made between genome organisation in a small genome species, i.e. rice, and large genome monocots such as grass, wheat and others; the determination of the genetic control of key traits; and provides a resource for gene isolation via a chromosome landing strategy, i.e. Introgression Landing.

Development of *Lp/Fp* substitution lines

The forage grass *Lolium perenne* (*Lp*) (2n=2x=14) can be readily hybridised with *Festuca pratensis* (*Fp*) (2n=2x=14) to form a 14-chromosome hybrid which exhibits full pairing at metaphase I of meiosis but nearly complete sterility (Lewis, 1966; Jauhar, 1975). However, *LpLpFp* triploids, derived by hybridising synthetic tetraploid *Lp* with diploid *Fp* show both male and female fertility. When these triploids are backcrossed to diploid *Lp* they give rise to BC_1 progeny containing 14 chromosomes (Figure 1). Although the majority of the genome of these individuals is derived from the *Lp* parent, over 74% carry one or more *Fp* chromosome segments. Most plants carry one or two introgressed *Fp* chromosome segments (King *et al*., 1998). Recombination, albeit at varying frequencies, has been observed to occur along the entire length of the chromosomes (I. King *et al*., 1998; 1999; J. King *et al.,* 2002a). In addition to individuals carrying *Lp/Fp* recombinant chromosomes, 14 chromosome plants carrying 13*Lp* chromosomes and 1 *Fp* chromosome, i.e. monosomic substitutions, have been isolated (Figure 1).

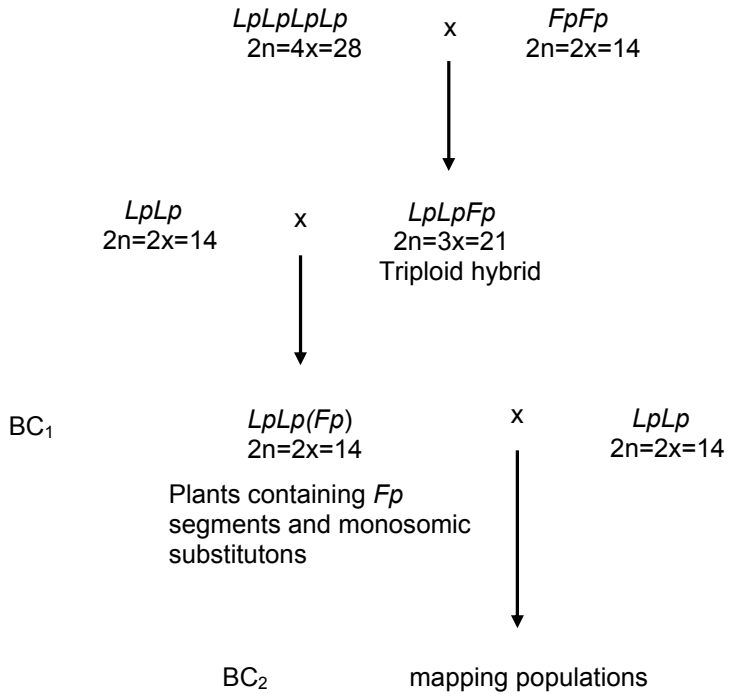

Figure 1 Crossing scheme for producing *Lolium/Festuca* introgressions and monosomic substituions

In order to isolate all seven possible substitution lines 550 BC_1 individuals were screened using RFLPs, AFLPs and SSR's (SSR's were derived from IGER's genomic microsatellites and from Vialactia's Gene Thresher library). All seven monosomic substitution lines have now been isolated and microsatellite analysis reveals that, at least at the macro-level, synteny has been maintained between *Lp* and *Fp.*

Genetic and introgression mapping of *Fp* chromosome 3

A single *Lp/Fp* monosomic substitution that carried a *Fp* chromosome homoeologous to the Triticeae group 3 chromosomes and rice chromosome 1 (King *et al*., 2002a) was selected for further research.

Development of a genetic map of Fp chromosome 3

The *Lp/Fp* group 3 monosomic substitution, which carries a nucleolar organiser region, was backcrossed to diploid *Lp* to produce a BC_2 mapping population (Figure 1). This population was analysed with AFLP's using two enzyme combinations (*Eco*R1/*Tru*91 and *Hin*dIII/*Tru*91). The initial AFLP analysis was carried out in order to find markers specific to the *Festuca* chromosome in the monosomic substitution (King *et al*., 2002a). To do this DNA from the four plants involved in the production of the substitution, i.e. *Fp*, *Lp* (tetraploid), *Lp* (diploid) and the *Lp/Lp/Fp* triploid hybrid, as well as the monosomic substitution itself, were screened. Markers found to be present in *Fp*, the triploid hybrid and the monosomic substitution, but absent from *Lp* (both the diploid and tetraploid genotypes) were classified as being specific to the *Fp* group 3 chromosome. Thirty five primer pairs generating 104 markers specific to *Fp* chromosome 3 were used to analyse 148 randomly selected plants. To keep scoring errors to a minimum all AFLP primer pairs/genotypes giving rise to singletons and questionable markers [as defined by the mapping programme – JOINMAP 2.0 (Stam, 1993)] were re-run and re-analysed at least once. This gave rise to a final genetic map of 81cM for the chromosome.

In addition to AFLP analysis the ability to distinguish the *Fp* chromosome in the *Lp/Fp* monosomic substitution line at meiosis using GISH enabled us to study the frequency of chiasmata in the *Lp/Fp* bivalent (King *et al*., 2002a). This analysis allowed us to predict the expected genetic length of the *Fp* chromosome derived from AFLP analysis. The predicted genetic length of the *Festuca* chromosome can be calculated from the mean number of chiasmata scored in the *Fp/Lp* bivalent (μ), visualised with GISH, using the expression 50μcM, For example, a chromosome with an average of two chiasmata is expected to have a genetic length of 50 x 2 = 100cM (Kearsey and Pooni, 1996). The average chiasma frequency for the *Lp/Fp* bivalent was 1.522 on the basis of 347 chiasmata in 228 PMCs. This value provided an estimate of the genetic length of the *Fp* chromosome using the expression 50μcM, i.e. 50 x 1.522 = 76cM. Thus the genetic linkage map of 81cM constructed from the AFLP markers was not significantly different from that predicted by chiasma frequencies, i.e. 76cM. This demonstrates a 1:1 correspondence between chiasma frequency and recombination rate (King *et al.,* 2002a). This result is in contrast to many reports (Nilsson *et al.*, 1993), which have shown large discrepancies between estimated genetic distances based on chiasma frequencies and genetic distances based on the segregation of genetic markers.

Development of an introgression map of Fp chromosome 3

The genetic linkage map of the *Fp* chromosome was used to select 16 plants from the BC_2 mapping population for physical mapping (King *et al*., 2002b). These plants were chosen because they showed a relatively even spread of recombination points along the chromosome and, where possible, recombination points on either side of the centromere and NOR.

Measurements taken of the recombinant chromosomes from mitotic root tip preparations in the BC_2 plants were: 1) total length of chromosome; 2) distance of recombination site or sites

from both telomeres; 3) position of the NOR site measured from the edge of the NOR nearest to the telomere. Measurements of *Lp/Fp* recombinant chromosomes were taken from enlarged projections of at least ten separate chromosomes for each of the BC_2 plants used for physical mapping. However, the *Lp* and *Fp* genomes differ in size with the *Fp* genome being 7% larger than the *Lp* genome (Bennett *et al*., 1982; Hutchinson *et al.*, 1979). Therefore the larger the *Fp* chromosome segment the larger the recombinant chromosome size and it is necessary to use an expansion factor to enable size comparisons of *Fp* segments in different genotypes to be made (King *et al.* 2002b).

The 16 BC_2 plants used for the physical mapping involved single crossovers (with the exception of BC_2 83). All the *Fp* segments observed extended from one or other of the telomeres. Thus two series of *Fp* segments were looked at using GISH: the first series increased in size from the telomere of the chromosome arm without the NOR, whilst the second series increased in size from the telomere of the chromosome arm carrying the NOR. This resulted in the *Fp* chromosome being split into 18 segments or bins (BC_2 83 contained two *Fp* segments which could be individually measured and mapped). The physical sites of recombination appeared to occur along the whole length of the chromosome including regions close to the centromere and within the NOR (King *et al.*2002b).

By combining the genetic and physical maps it has been possible to assign each of the AFLPs used to genetically map chromosome 3 to one of the 18 physical bins. This work has allowed comparisons to be made between genetic and physical distance.

Two gaps of greater than 10% of the chromosome arm were observed on the physical map of the *Fp* chromosome. The distribution of recombination sites along the whole length of the chromosome, however, shows that the present physical map has the potential to be broken down into much smaller sections by screening large populations for recombination between two markers that flank a specific region of the genome. A comparison of the physical and genetic maps clearly shows how their inter-relationship varies from one part of the chromosome to another. The two gaps on the physical map do not coincide with gaps on the genetic map. In fact, the density of AFLP markers on the genetic map is such that the largest distance between markers is only 5.9cM and there are only two other gaps of between 4 and 5cM present. Of the two gaps on the physical map, the first (a gap of 15.2% of the chromosome) contains 11 AFLP markers spread over just 1.3cM, while the second (a gap of 13.4% of the chromosome) contains eight AFLP markers spread over 9.1cM.

The distribution of recombination sites along the whole length of the chromosome included those very close to the centromere and within the NOR although not between the two. Thus, although the centromere and NOR both cause a reduction in the frequency of recombination in the region between them (see below), recombination itself does take place within these regions.

Recombination levels were found to vary within, as well as between arms. The highest frequency of recombination occurred at a physical distance of between 12 and 20% from the telomeres. The lowest frequency was found between 45 and 75% of the distance along the chromosome (the region of the chromosome containing both the centromere and the NOR).

Our results show that the centromere was physically mapped at 49.2% of the distance along the chromosome. The frequency of recombination started to increase at a distance of only about 5% from the centromere in the arm without the NOR. In contrast, it remained extremely

low in the NOR arm for the whole of the region between the centromere and the NOR and including the NOR itself, but rose sharply after the end of the NOR. However, the peak in the NOR arm was considerably smaller than the major peak in the non-NOR arm. This result strongly suggests that the NOR, as well as the centromere, causes a reduction in the frequency of recombination. Similar evidence for little or no crossing over between the centromere and NOR has been reported for chromosomes 1B and 6B of wheat (Payne *et al*., 1984; Dvořák and Chen, 1984; Snape *et al*., 1985), barley chromosomes 6 and 7 (Linde-Laursen, 1979) and rye chromosome 1R (Lawrence and Appels; 1986).

The results obtained from this work are in agreement with data obtained from a range of other species where it has been shown that there is not a consistent relationship between genetic distance in cM and physical distance in base pairs, and that there is variation in this relationship from one part of the genome to another, e.g. Gustafson and Dillé (1992), CHEN and Gustafson (1995), Werner *et al.* (1992), Hohmann *et al*. (1994), Hohmann *et al*. (1995), Delaney *et al.* (1995), Mickelson-Young *et al.* (1995), Gill *et al.* (1996a and b), Künzel *et al*. (2000). Genetically close markers may actually be far apart in terms of base pairs (or vice versa) due to differences in the frequency of recombination along the length of a chromosome. When considering the average length of DNA per unit of recombination, different segments of a chromosome should therefore be considered independently. For chromosome 4 of *Arabidopsis,* the base-pair to cM ratio varied from 30kb to 550kb per cM (Schmidt *et al*., 1995). In rice 1cM is on average equal to 240kb, although this figure actually varies from 120 to 1000kb per cM (Kurata *et al*., 1994). In wheat the variation is even more extreme, with 1cM equal to 118kb in regions of high recombination but 22000kb in regions of low recombination (Gill *et al*., 1996a and b). Regions corresponding to centromeres, and even some telomeres in tomato and potato, show a ten-fold decrease in recombination compared to other regions in the genome (Tanksley *et al*., 1992). Reduced recombination frequency in pericentric regions is also seen in many species including the grasses, e.g. wheat (Dvořák and Chen, 1984; Snape *et al*., 1985; Curtis and Lukaszewski, 1991; Gill *et al*., 1993 and 1996a and b; for review see Gill and Gill, 1994), barley (Leitch and Heslop-Harrison, 1993; Pedersen *et al*., 1995; Künzel *et al.*, 2000), rye (for review see Heslop-Harrison, 1991; Wang, 1992), *Lolium* (Hayward *et al.*, 1998; Bert *et al.,* 1999). Nucleolar organiser regions (NORs) may also cause a reduction in the frequency of crossing over, e.g. *Allium schoenophrasum* (J.S. Parker pers. com.). Recombination hotspots also occur (Endo and Gill, 1996; Weng *et al.,* 2000; Künzel *et al*., 2000).

Thus in the *Lp/Fp* work described here genes located on the *Fp* chromosome arm without the NOR will appear genetically much further apart than genes located on the chromosome arm carrying the NOR. However, the genetic distance between the genes on the two chromosome arms will have very little relevance with regard to the physical distance between genes.

Development of an introgression map of the *Lp/Fp* genomes

Festuca chromosome 3 as been used as a prototype for the development of an introgression map of the *Lp/*Fp genomes. Each of the six remaining *Lp/Fp* monosomic substitution lines have been backcrossed to diploid *Lp* to generate BC_2 mapping populations. As with the monosomic substitution carrying *Fp* chromosome 3, the *Lp* and *Fp* homoeologous bivalents in the other monosomic substitution lines undergo high frequencies of recombination along the length of the chromosomes during meiosis, resulting in the generation of *Lp/Fp* recombinant chromosomes. The mapping populations derived from each of these seven substitutions are presently being screened with microsatellite markers (500 SSRs;

IGER/Vialatia) which will enable the genetic mapping of each of the seven substituted *Festuca* chromosomes. These genetic marker profiles are being used to identify individuals, derived from each of the seven mapping populations, which carry *Lp/Fp* recombinant chromosomes with different sized and overlapping *Fp* chromosome segments. The physical size and position of the overlapping *Fp* segments in each of the recombinant chromosomes will then be measured using GISH. These data will then be assembled enabling each of the seven *Festuca* chromosomes to be divided into introgression bins, i.e. each of the seven *Festuca* chromosomes will initially be divided into at least 20 bins composed of no more than 5% of the total chromosome length. However, the resolution of the system is such that it is possible to divide the chromosomes into much smaller bins, i.e. at least 0.4% of the total chromosome length.

Exploitation of the *Lolium/Festuca* introgression maps

A programme at IGER has recently been initiated to bin map every 5th rice BAC. The bin mapping of rice BACs requires that a coding region is identified on each BAC that is to be mapped. This sequence is then used to screen other monocot databases. Primers are designed from conserved regions and these are used to amplify the equivalent sequence in *Lp* and *Fp* via PCR. The products are sequenced and SNP markers that discriminate between *Lp* and *Fp* are designed. Once identified, SNPs can be mapped to a specific bin on a specific chromosome (a similar strategy will be applied using orthologous markers from other monocots such as barley). This strategy is proving successful for *Fp* chromosome 3. At the time of writing 24 rice BACs have been mapped to *Fp* chromosome 3.

Bin mapping rice BACs will enable the determination of the organisation of genes in large genome plant species, such as *Fp*, and allow comparisons to be made with the small genome model, rice. The work will allow determination of the distribution of genes along the *Fp* chromosomes. Are genes (I) evenly distributed along the chromosome and separated by large regions of repetitive DNA? or (II) present in clusters surrounded by repetitive DNA? If clustered, how are the clusters themselves organised: are they evenly distributed or are the clusters clustered? In addition, the work will allow the elucidation of the frequency and distribution of recombination relative to gene density, i.e. do peaks in recombination frequency coincide with gene rich areas? Are some genes or clusters of genes located in areas of low recombination and if so has this led to the development of co-adapted gene complexes which confer a selective advantage? A knowledge of the relationship between recombination and the physical location of target genes is of importance since the success of both conventional breeding programmes and gene isolation, via forward genetics, are heavily dependent on the frequency of recombination in the region in which a target gene is located. Our initial results indicate that the majority of rice BACs are located in areas of low recombination.

Introgression mapping will also facilitate the exploitation of information and technology developed in the model plants. The rice genome project has provided the order and sequence of the genes on each of its 12 chromosomes. This data will enable the isolation of genes, via chromosome landing and walking strategies, from large genome monocot species. For example EST based markers that flank a target gene in a large genome crop species are being used to identify the equivalent region in rice. Since the rice genome has been sequenced, every gene between the flanking markers in this species is known. These rice gene sequences can be used to develop additional markers which will then be used to isolate the target gene via chromosome landing approaches in the crop species itself. However, this approach

assumes that the gene order in rice and other monocot crop species is the same. The bin mapping of rice BACs will provide an in depth genome wide assessment of the syntenic relationship between rice and *Lp* and *Fp*. In addition, the maintenance of synteny between rice and grass is likely to indicate a similar relationship between other monocots and rice. Thus the *Lp/Fp* introgression mapping described will benefit the whole monocot research community. In addition, strategies are presently being developed where by introgression maps in combination with other technologies will provide a platform to contig the *Lp/Fp* genomes.

The monosomic substitutions and the individuals making up the physical maps of each *F. pratensis* chromosome will also provide a valuable resource for determining the genetic control of target traits and gene isolation. For example, the seven monosomic substitution lines will be screened for a specific trait. Once a *F. pratensis* chromosome has been identified as carrying a gene(s) controlling the trait the relevant genotypes making up the physical map will also be screened. In this way it will be possible to physically map genes that control key traits. Furthermore, physical mapping/bin mapping will identify syntenic regions in rice thus facilitating the isolation of genes responsible for the control of a trait via map based cloning.

A strategy for the isolation of introgressed *Fp* genes

The unique combination of genetic and cytogenetic characteristics exhibited by *Lp/Fp* and also *Lm/Fp* hybrids and their derivatives (the chromosomes undergo a high frequency of homoeologous recombination at meiosis; the chromosomes of the two species can easily be discriminated by GISH; recombination occurs along the entire length of homoeologous bivalents; a high frequency of marker polymorphism is observed between the two species) are being exploited to isolate tightly linked markers to target genes and to provide a springboard for gene isolation.

A *Fp* chromosome segment which carries a mutation of a gene normally required for leaf yellowing during senescence has been introgressed into *Lm* (Thomas *et al*., 1987 and Thomas *et al*., 1997). The stay-green character results from a recessive mutation in the gene, and only plants homozygous for the mutation express the stay-green phenotype. Leaf segments of plants homozygous for the mutation remain green, while plants heterozygous or homozygous for the wild type gene turn yellow as chlorophyll is broken down. The lesion in the chlorophyll breakdown pathway in plants homozygous for the green gene appears to result from the inability of plants to break down pheophorbide to red-chlorophyll-catabolite (RCC) because of a deficiency in pheophorbide-a-oxygenase (PaO) activity (Vicentini *et al.,* 1995; Rodoni *et al.,* 1997; Thomas *et al.,* 2001). Thus the stay green phenotype is believed to result from a mutation in the gene responsible for the production of the PaO enzyme or a regulator gene that controls the expression or activation of the gene/ protein (Roca *et al*., 2004).

A mapping family was generated from the *Lm/Fp* introgression line carrying the stay-green mutation and AFLP's were used to generate a genetic map of the *Fp* chromosome segment (Moore *et al*., 2005). AFLP analysis was performed, using the restriction enzyme pairs *Hin*dIII/*Tru*91 and *Eco*R1/*Tru*91. Polymorphisms specific to the *Fp* segment were identified by screening the parents, i.e. tetraploid *Lm*, diploid *Fp*, diploid *Lm*, the *Lm/Lm*/*Fp* triploid hybrid and the selected BC_1 genotype carrying a single small *Fp* chromosome segment. Primer pairs which failed to give a *Fp* specific polymorphism or primer pairs which gave a *Fp* specific polymorphism in the *Fp* parent and *Fp* hybrid but were not in the selected BC_1 introgression genotype, i.e. those where the *Fp* specific marker lay outside the introgressed *Fp* chromosomes

segment, were discarded. Primer pairs which gave a *Fp* specific polymorphism in the *Fp* diploid parent, the *Lm/Lm/Fp* triploid and the selected BC_1 individuals were selected.
Twenty-two selected AFLP primer pairs, giving 28 *Fp* specific polymorphisms (Figure 2a), were used to screen the mapping population. The segregation of the *Fp* specific polymorphisms in the mapping population was analysed using JOINMAP™ 2.0 (Stam, 1993) to generate a genetic map of the *Fp* chromosome segment (Figure 2a and figure 2b). Each of the individuals of the BC_2 mapping population was also test-crossed to a *Lm* genotype homozygous recessive for the green gene (yy) to determine the presence or absence of the stay green gene in each of the individuals of the mapping population. The data for the presence or absence of the stay-green gene were combined with the AFLP data in order that the senescence mutation could be mapped within the introgressed *Fp* chromosome segment. The final genetic distance of the *Fp* chromosome segment between the terminal *Fp*-derived AFLP markers was estimated to be 19.8cM with the stay-green *sid* mutation located at 9.8cM; the closest flanking markers to *sid* were at 0.6cM and 1.3cM.

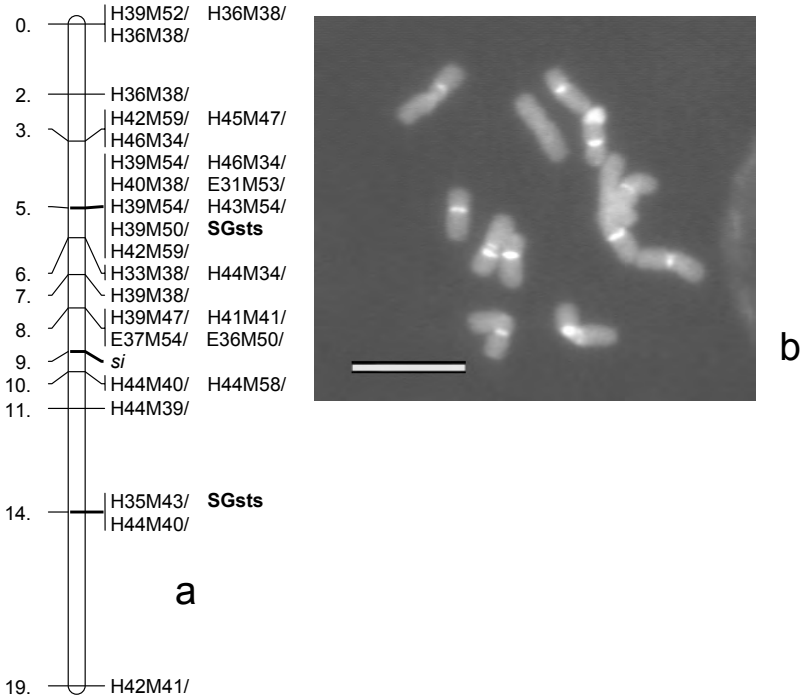

Figure 2a Linkage group of introgressed *F. pratensis* segment containing the stay-green gene (*sid*). Genetic distances are indicated in centiMorgans. The positions of STS markers derived from AFLP/BAC sequencing are indicated in bold-type; the positions of AFLP markers are indicated in normal-type.
Figure 2b BC_1 plant showing the smallest introgressed segment from *F.pratensis* carrying the stay green gene (sid)

Twelve AFLP bands were excised, cloned and sequenced. Primers designed from one of these 12 AFLPs (SG7), which produced a *Fp* specific fragment of 390 bp, immediately

distinguished between the *Lolium* and *Festuca* genotypes, the other 11 did not. Most of the internal sequences of the other markers were not polymorphic between the parents and therefore not useful for conversion to STS markers. Therefore a *Fp* BAC library (Donnison *et al*., 2005) was screened with AFLP-derived primers from another two markers on the other side of the stay-green locus, with the aim of identifying additional sequences suitable for designing new primers. One primer pair identified many BACs indicating that the sequence was present in multiple locations in the genome, but the other (SG2), which generated a *Fp* AFLP fragment of 300 bp, identified two. Given the 2.5x coverage of the BAC library, this fragment was considered to be present as a single copy sequence. BAC DNA of an individual BAC identified from this marker was extracted, digested and re-cloned. End-sequencing of these BAC sub-clones generated 7 Kb of additional sequence. This sequence was used to generate an extra primer pair from a BAC sub-clone fragment which did not show homology to repetitive DNA such as retroelements of other monocot species. This primer pair was tested on *Lolium* and *Festuca* parental DNA and shown to be polymorphic. Primer pairs for this and the other polymorphic marker were then mapped back onto the genetic map and in both cases were found to map precisely to the same position as the original AFLP markers, on either side of the stay-green locus (Figure 2a).

The work described above makes use of *Fp* specific AFLP polymorphisms to map an alien chromosome segment. An alternative strategy will be to derive markers from the sequenced rice genome. Once the region of the rice genome that shows synteny with a *Fp* chromosome segment has been identified, additional markers for the introgressed segment can be developed. This can be achieved by comparing a predicted coding sequence from rice with EST databases from other monocots. Primers can then be developed from regions that show very high conservation. Ninety per cent of such primers have been shown to generate an equivalent sequence in *Lolium* and *Festuca* and a high proportion show polymorphism between the two species. The advantage of this strategy is that it provides large numbers of markers for a specific region of the *Fp* genome that is of interest, as well as possible information on gene function in the model monocot plant species. The potential of isolating *Fp* genes via the use of large numbers of rice markers and high resolution *Lolium*/*Fp* mapping populations is presently being investigated. A large mapping population of over 2000 individuals has been mapped with markers, derived from rice, that closely flank the green gene. This procedure '*Introgression Landing*' has identified a region of the rice genome that carries 30 genes and these will be functionally tested to determine which of these is responsible for the stay-green phenotype. In addition to this, markers that discriminate between *Lp, Lm* and *Fp* have been developed for use in IGER breeding programmes.

Our initial research indicates that *Introgression Landing*, in combination with robust screening procedures for target traits provides a fast and efficient method of isolating closely linked markers to target genes and provides a platform for gene isolation.

Acknowledgements

Julie King is supported by an EMBO Restart Fellowship. Beverley Moore was supported by the Teaching Company Scheme. IGER is sponsored by the BBSRC.

References

Bennett, M.D., J.B Smith & J.S. Heslop-Harrison (1982). Nuclear DNA amounts inangiosperms. *Proc. Royal Soc.* London B., 216**,** 179-199.

Bert, P.F., G.Charmet, P. Sourdille, M.D. Hayward & F. Balfourier (1999). A high density molecular map for ryegrass (*Lolium perenne*) using AFLP markers. *Theor. Appl. Genet.*, 99, 445-452.
Chen, J.M. & J.P. Gustafson (1995). Physical mapping of restriction fragment length polymorphisms (RFLPs) in homoeologous group 7 chromosomes of wheat by *in situ* hybridisation. *Heredity*, 75, 225-233.
Curtis, C.A. & A.J. Lukaszewski (1991). Genetic linkage between C-bands and storage protein genes in chromosome 1B of tetraploid wheat. *Theor. Appl. Genet.*, 81, 245-252.
Delaney, D.E., S. Nasuda, T.R. Endo, B.S. Gill & S.H. Hulbert (1995). Cytogenetically based physical maps of the group 3 chromosomes of wheat. *Theor. Appl. Genet.*, 91, 780-782.
Donnison, I.S., D. O'Sullivan, A. Thomas, P. Canter, B.J. Moore, I.P. Armstead, H. Thomas, K. Edwards & I.P. King (2005). Construction of a *Festuca pratensis* BAC library for map based cloning in *Festulolium* substitution lines. *Theor. Appl. Genet.*, in press.
Dvořák, J. & K-C. Chen (1984) Distribution of nonstuctural variation between wheat cultivars along chromosome arm 6Bp: evidence from the linkage map and the physical map of the arm. *Genetics*, 106, 325-333.
Endo, T.R. & B.S. Gill (1996). The deletion stocks of common wheat. *J. Hered.*, 87, 295-307.
Gill, K.S., B.S. Gill & T.R. Endo (1993). A chromosome region specific mapping strategy reveals gene rich telomeric ends in wheat. *Chromosoma*, 102, 374-381.
Gill, K.S. & B.S. Gill (1994). Mapping in the realm of polyploidy: the wheat model. *BioEssays*, 16, 841-846.
Gill, K.S., B.S. Gill, T.R. Endo & E.V. Boyko, (1996a) Identification and high density mapping of gene rich regions in chromosome group 5 of wheat. *Genetics*, 143, 1001-1012.
Gill, K.S., B.S. Gill, T.R. Endo & T. Taylor (1996b). Identification and high-density mapping of the gene-rich regions in chromosome group 1 of wheat. *Genetics*, 144, 1883-1891.
Gustafson, J.P. & J.E. Dillé (1992). Chromosome location of *Oryza sativa* recombination linkage groups. *Proc. Natl. Acad. Sci. USA*, 89, 8646-8650.
Hayward, M.D., J.W. Forster, J.G. Jones, O. Dolstra, C. Evans, (1998). Genetic analysis of *Lolium*. I. Identification of linkage groups and the establishment of a genetic map. *Plant Breed.*, 117, 451-455.
Heslop-Harrison, J.S. (1991). The molecular cytogenetics of plants. *J. Cell Sci.*, 100, 15-21.
Hohmann, U., T.R. Endo, K.S. Gill & B.S. Gill (1994). Comparison of genetic and physical maps of group 7 chromosomes from *Triticum aestivum* L. *Mol. Gen. Genet.*, 245, 644-653.
Hohmann, U., A. Graner, T.R. Endo, B.S. Gill & R.G. Herrmann (1995). Comparison of wheat physical maps with barley linkage maps for group 7 chromosomes. *Theor. Appl. Genet.*, 91, 618-626.
Hutchinson, J., H. Rees & A.G. Seal (1979). An assay of the activity of supplementary DNA in *Lolium*. *Heredity* 43, 411-421.
Jauhar, P.P. (1975). Chromosome relationships between *Lolium* and *Festuca* (Graminea). *Chromosoma*, 52, 103-121.
Kearsey M.J. & S. Pooni (1996) The genetical analysis of quantitative traits, pp116-119, Publishers, Chapman and Hall, London.
King, I.P., W.G. Morgan, I.P. Armstead, J.A. Harper, M.D. Hayward, A. Bollard, J.V. Nash, J.W. Forster & H.M. Thomas (1998). Introgression mapping in the grasses I. Introgression of *Festuca pratensis* chromosomes and chromosome segments into *Lolium perenne*. *Heredity*, 81, 462-467.
King, I.P., W.G. Morgan, J.A. Harper & H.M. Thomas, (1999). Introgression
mapping in the grasses. II. Meiotic analysis of the *Lolium perenne/Festuca pratensis* triploid hybrid. *Heredity*, 82**,** 107-112.
King J, L.A. Roberts, M.J. Kearsey, H.M. Thomas, R.N. Jones, L. Huang, I.P. Armstead, W.G. Morgan, & I.P. King (2002a). A demonstration of a 1:1 correspondence between chiasma frequency and recombination using a *Lolium perenne/Festuca pratensis* substitution line. *Genetics,* 161, 315-324.
King J, I.P. Armstead, I.S. Donnison, H.M. Thomas, R.N. Jones, M.J. Kearsey, L.A. Roberts, A. Jones, & I.P. King (2002b). Physical and genetic mapping in the grasses *Lolium perenne* and *Festuca pratensis*. *Genetics,* 161, 315-324.
Künzel, G., L. Korzun & A. Meister (2000). Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. *Genetics*, 154, 397-412.
Kurata, N., Y. Nagamura, K. Yamamoto, Y. Harushima, & N. Sue. (1994). A 300 kilobase interval genetic map of rice including 883 expressed sequences. *Nature Genetics*, 8, 365-372.
Lewis, E.J. (1966). The production and manipulation of new breeding material in *Lolium-Festuca*. In: Hill, AG.G. (ed) Proc. X Int. Grassland Congr., Valtioneuvoston Kirjapaino, Helsinki, pp 688-693.
Lawrence, G.J. & R. Appels (1986). Mapping the nucleolar organiser region, seed protein loci and isozyme loci on chromosome 1R in rye. *Theor. Appl. Genet.*, 71, 742-749.
Leitch, I.J. & J.S. Heslop-Harrison (1993). Physical mapping of 4 sites of 5s rRNA sequences and one site of the *α-amylase-2* gene in barley (*Hordeum vulgare*). *Genome*, 36, 517-523.

Linde-Laursen, I. (1979) Giemsa C-banding of barley chromosomes III. Segregation and linkage of C bands on chromosomes 3, 6 and 7. *Hereditas*, 91, 73-77.

Mickelson-Young, L., T.R. Endo & B.S. Gill (1995). A cytogenetic ladder map of the wheat homoeologous group 4 chromosomes. *Theor. Appl. Genet*,. 90, 1007-1011.

Moore, B.J., I.S. Donnison, J.A. Harper, I.P. Armstead, J. King, H. Thomas, R.N. Jones, T.H. Jones, H.M. Thomas, W.G. Morgan, A. Thomas, H.J. Ougham, L. Huang, T. Fentem, L.A. Roberts & I.P. King (2005). Molecular Tagging of a senescence gene by introgression mapping of a stay-green mutation from *Festuca pratensis. New Phytologist*, 165, 801-806.

Nilsson N-O., T. SÄll & B.O. Bengston (1993) Chiasma and recombination data in plants: are they compatible? *Trends Genet.*, 9**,** 344-348.

Payne, P.I., L.M. Holt, J. Hutchinson & M.D. Bennett, (1984) Development and characterisation of a line of bread wheat, *Triticum aestivum*, which lacks the short arm satellite of chromosome 1B and the *Gli-B1* locus. *Theor. Appl. Genet.* 68, 327-334.

Pedersen, C., H. Giese & I. Linde-Lauresen (1995). Towards an integration of the physical and the genetic chromosome map of barley by *in situ* hybridisation. *Hereditas*, 123, 77-88.

Roca, M., C. James, A. Pružinská, S. Hortensteiner, H. Thomas, & H. Ougham (2004). Analysis of the chlorophyll catabolism pathway in leaves of an introgression senescence mutant of Lolium temulentum. *Phytochemistry*, 65, 1231-1238.

Rodoni S, W. Muhlecker, M. Anderl, B. Krautler, D. Moser, H. Thomas, P. Matile, & S. Hortensteiner (1997). Chlorophyll breakdown in senescent chloroplasts - cleavage of pheophorbide a in two enzymic steps. *Plant Physiology*, 115,669-676.

Schmidt, R., J. West, K. Love, Z. Lenechan, C. Lister (1995). Physical map and organisation of *Arabidopsis thaliana* chromosome 4. *Science*, 270, 480-483.

Snape, J.W., R.B. Flavell, M. O'Dell, W.G. Hughes & P.I. Payne (1985). Intrachromosomal mapping of the nucleolar organiser region relative to three marker loci on chromosome 1B of wheat (*Triticum aestivum*). *Theor. Appl. Genet.,* 69**,** 263-270.

Stam, P. (1993). Construction of integrated genetic linkage maps by means of a new computer package. JOINMAP. *Plant J.*, 3, 739-744.

Tanksley, S.D., M.W. Ganal, J.P. Prince, M.C. deVicente, & M.W. Bonierbale (1992). High density linkage maps of the tomato and potato genomes. *Genetics*, 132, 1141-1160.

Thomas H. (1987). *sid*: A Mendelian locus controlling thylakoid membrane disassembly in senescing leaves of *Festuca pratensis*. *Theor Appl Genet*, 73, 551-555.

Thomas H, C. Evans, H.M. Thomas, M.W. Humphreys, W.G. Morgan, B.D. Hauck, I. S. Donnison (1997). Introgression, tagging and expression of a leaf senescence gene in *Festulolium. New Phytologist*, 137, 29-34.

Thomas H, H. J. Ougham, S. Hortensteiner (2001). Recent advances in the cell biology of chlorophyll catabolism. *Advances in Botanical Research*, 35, 1-52

Vicentini F., S. Hortensteiner, M. Schellenberg, H. Thomas and P. Matile (1995). Chlorophyll breakdown in senescent leaves: identification of the biochemical lesion in a stay-green genotype of *Festuca pratensis*. *New Phytologist*, 129, 247-252.

Wang, M.L., M.D. Atkinson, C.N. Chinoy, K.M. Devos & M.D. Gale (1992). Comparative RFLP-based genetic maps of barley chromosome 5 (1H) and rye chromosome 1R. *Theor. Appl. Genet.*, 84, 339-344.

Weng, Y., N.A. Tuleen & G.E. Hart (2000). Extended physical maps and a consensus physical map of the homoeologous group 6 chromosomes of wheat (*Triticum aestivum* L. em Thell). *Theor. Appl. Genet*,. 100, 519-527.

Werner, J.E., T.R. Endo & B.S. Gill (1992) Toward a cytogenetically based physical map of the wheat genome. *Proc. Natl. Acad. Sci. USA*, 89, 11307-11311.