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A high-density molecular map for ryegrass (*Lolium perenne*) using AFLP markers

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Abstract AFLP markers have been successfully employed for the development of a high-density linkage map of ryegrass (*Lolium perenne* L.) using a progeny set of 95 plants from a testcross involving a doubled-haploid tester. This genetic map covered 930 cM in seven linkage groups and was based on 463 amplified fragment length polymorphism (AFLP) markers using 17 primer pairs, three isozymes and five EST markers. The average density of markers was approximately 1 per 2.0 cM. However, strong clustering of AFLP markers was observed at putative centromeric regions. Around these regions, 272 markers covered about 137 cM whereas the remaining 199 markers covered approximately 793 cM. Most genetic distances between consecutive pairs of markers were smaller than 20 cM except for five gaps on groups A, C, D, F and G. A skeletal map with a uniform distribution of markers can be extracted from this high-density map, and can be applied to detect and map QTLs. We report here the application of AFLP markers to genome mapping, in *Lolium* as a prelude to quantitative trait locus (QTL) identification for diverse agronomic traits in ryegrass and for marker-assisted plant breeding.

Key words Genetic mapping · AFLP · *Lolium perenne*, Plant breeding

Introduction

The genus *Lolium* includes eighet species (Terrell 1968). Among them, two major fodder crops are cultivated for temperate grassland agriculture: perennial ryegrass (*Lolium perenne*) for grazing and turf and Italian ryegrass (*L. multiflorum*) for hay and silage making. *L. perenne* is one of the most important forage grasses commonly cultivated in Europe, New Zealand, Japan and Australia for permanent pasture and amenity grassland. Until recently, forage plant breeding was carried out with limited insight into the genotypic breeding value. Grass breeders have recognised the potential of DNA markers to enable events at the genome level to be understood and monitored in a much more precise way (Hayward et al. 1994). As genetic maps facilitate the study of genome structure and evolution, as well as the identification of monogenic traits or Mendelian components of quantitative traits (QTLs), they can be very useful in current breeding programs and are also the basis for future positional gene cloning. Substantial effort is currently being directed towards the production of genetic maps in many grass species. Genetic mapping of *Lolium* species (2n=14) has been relatively little developed compared to the cereals such as wheat (Chao et al. 1989; Devos and Gale 1993) or rice (McCouch et al. 1988; Maheswaran et al., 1997) probably because of its lower economical importance and its relatively large genome size (2 C=4.16 pg, Hutchinson et al. 1979; 1660 Mbp, Forster 1999). Nevertheless, a *Lolium* linkage map based on an interspecific cross (*L. perenne*×*L. multiflorum*) has already been constructed (Hayward et al.1994) and recently extended to include 106 markers distributed over 692 cM (Hayward et al. 1998).

Restriction fragment length polymorphisms (RFLPs; Botstein et al. 1980) have been extensively used for the construction of genetic linkage maps in plants. However, they require large amounts of genomic DNA, are time consuming and expensive. Since the development of the polymerase chain reaction (PCR) for amplifying DNA fragments (Saiki et al. 1988), several PCR-based technologies have been developed. Random amplified polymorphic DNA (RAPD; Williams et al. 1990) and microsatellite (Litt and Luty 1989; Roder et al. 1998) markers overcome many of the technical limitations of RFLPs but have their own limitations: RAPDs have the major

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disadvantage of being dominant and very sensitive to the reaction conditions, while microsatellites are expensive to produce. More recently, a new PCR-based-technology has been developed by Vos et al. (1995), termed amplified fragment length polymorphism (AFLP), which uses a PCRs system to detect RFLP indirectly. AFLP methodology takes advantage of the polymerase chain reaction but differs from classical PCR and single primer-based methods such as RAPD, AP-PCR (arbitrary primed PCR; Welsh and McClelland 1990) and DAF (DNA amplified fragments; Caetano-Anolles et al. 1991) by generating highly reproducible multi-banded profiles which can reveal extensive polymorphism, resulting in reduced work loads and increased speed of data generation. The AFLP technique has the capacity to reveal a much greater number of polymorphic loci than other currently available techniques (a virtually unlimited number of fragments can be detected) and is particularly amenable for genetic mapping and profiling studies (Karp et al. 1996).

The objective of the present study was to extend the genetic map for ryegrass exploiting an intraspecific population and the use of isozyme, EST (expressed sequence tags; Adams et al. 1991) and AFLP markers. The population involved is one being used by the International *Lolium* Genome Initiative (ILGI, Forster 1999) as a common basis for a co-ordinated international programme on genome analysis in the genus *Lolium*. The linkage map reported in this paper contributes to our knowledge of the genome structure of ryegrass and may be exploited as a potential reference map for QTL analysis as part of the ILGI programme.

Materials and methods

Plant material

An intraspecific (*L. perenne)* population (coded p150/112) of 150 plants was constructed at the Institute of Grassland and Environmental Research, Aberystwyth. It derived from a cross between a di-haploid plant (DH290) and a hybrid F_1 [Romanian collection no.Ba 9982× (a plant from a polycross of a North Italian collection of perennial ryegrass×th cv Melle)]. A subset of 95 plants, randomly selected, were used for marker screening and map construction.

Preparation of genomic DNA and AFLP reactions

Approximately 0.5 g of young leaves was harvested and ground in liquid nitrogen to obtain a fine powder. The CTAB method of Rogers and Bendich (1985) was used for the isolation of ryegrass genomic DNA. The AFLP protocol developed by Vos et al. (1995) was followed with minor modifications. Genomic DNA (350 ng) was restricted with 5 units of *Eco*RI and *Tru*9I at 37 C for 3 h according to the manufacturer's instructions (Boehringer Mannheim). *Tru*9I is an isoschizomer of *Mse*I. After complete digestion, 5 pMol of the *Eco*RI adaptor (5′-CTC GTA GTA GAC TGC GTA CC; CTG ACG CAT GGT TAA-5′), 50 pmol of the *Tru*9I adaptor (5′-GAC GAT GAG TCC TGA G; TAC TCA GGA CTC AT-5′; Zabeau and Vos 1993), 1 unit of T4 DNA Ligase and ligation buffer (Gibco BRL Life Technologies) were added and the mixture incubated for 2 h at 23 C. Pre-amplification was performed with *Eco*RI and *Tru*9I primers having one selective nucleotide. The pre-amplification reaction was conducted with 30 ng each of *Eco*RI and *Tru*9I single-nucleotide selective primers, 5 μl of 1:10-diluted ligated DNA, 1 unit of *Taq* DNA polymerase, 1× PCR buffer and 0.2 mM of dNTPs (Boehringer Mannheim). Pre-amplification PCR-cycle profiles were performed as described by Vos et al. (1995) with a 10-min final extension cycle at 72 C. Five microliters of a 1:50 diluted pre-amplified DNA was selectively amplified using 30 ng each of *Eco*RI and *Tru*9I primers with three selective nucleotides, 1 unit of *Taq* DNA polymerase, 1× PCR buffer and 0.2 mM of dNTPs using the PCR-cycle profile described by Vos et al. (1995) with a 10-min final extension cycle at 72 C. All PCR reactions were performed using a 9600 Perkin Elmer thermocycler.

Gel electrophoresis

Two microliters of each sample together with 1 μl of loading buffer (95% formamide, 10 mM NaOH, 0.05% each of xylene cyanol and bromophenol blue) were loaded after denaturation (5 min at 95 C) onto a 5% denaturing polyacrylamide gel (8 M urea; 50cm×0.4 mm) and electrophoresed at 2,000 V for 3.5 h in 1× TBE buffer (Tris 90 mM, pH 8.2, Borate 90 mM, EDTA 2 mM). PCR products were visualised using the silver-nitrate staining method as described by Tixier et al. (1997).

Isozyme and EST analysis

Three isozyme markers, phosphoglucomutase (PGM, EC 5.4.2.2), glutamic-oxaloacetic transaminase (GOT, EC 2.6.1.1) and acid phosphatase (ACP, EC 3.1.3.2), were analysed for mapping using the methods described by Hayward et al. (1995).

The five EST markers corresponding to OSE (Late Embryogenesis Abundant gene), OSRB (α-amylase), OSW (ADP-glucose glycosyl transferase), LP1 (pollen allergen) and MZE (triosephosphate isomerase) were analysed according to Lallemand et al. (1998). As for AFLPs the EST polymorphism was detected directly as a size difference.

Data analysis and map construction

AFLP markers were identified based on the primer-pair combination employed used and the estimated molecular size. The approximate size of each marker was expressed in nucleotides as estimated in comparison with the mobility of the bands of the 10-base ladder (Sequamark, Research Genetics). Clearly readable AFLP bands were scored as dominant genetic markers. The software package JOINMAP 2.0 (Stam 1993) was used to estimate segregation distortion and determine linkage groups, while the software program MAPMAKER 3.0b (Lander et al. 1987) was used to order loci and construct linkage maps. Analyses were performed with a LOD score threshold of 5.0 and a maximum recombination value of 25% (=0.25) for grouping and ordering markers. Kosambi's mapping function was applied for the calculation of map distances (Kosambi 1944).

Results

DNA marker generation

An investigation of the reliability of markers was first determined through a reproducibility test at each step in the AFLP procedure from DNA extraction to final selective amplification. Variation in the banding pattern generated was found to be in the range of 1–2%, (i.e. 1–2 additional or missing amplified DNA fragments over the total number of DNA fragments amplified). As already

Table 1 Total number of AFLP markers generated per primer pair using 17 *Eco*R1+3/*Tru*9I+3 primer combinations, including skewed markers, unlinked markers (P<0.001) and mapped markers, with their assignment to individual linkage groups

Primer pairs		Total markers	Skewed markers	Unlinked markers	Mapped markers	Linkage groups						
						A	B	C	D	E	F	G
E33(AAG)	T48(CAC)	32	0	1	31	4	7	5	4	3	5	3
E33 (AAG)	T50 (CAT)	35	0	3	32	2	4	5	6	4	3	8
E33 (AAG)	T62 (CTT)	35	1	2	32	8	10	5	2	1	3	3
E33 (AAG)	T61 (CTG)	32	0	2	30	5	3	8	1	2	5	6
E35 (ACA)	T50 (CAT)	19	1	1	17	1	4	4	4	0	2	2
E35 (ACA)	T59 (CTA)	21	2	2	17	7	3	3	2	0	1	1
E36 (ACC)	T47 (CAA)	34	0	0	34	4	3	8	5	3	4	7
E36 (ACC)	T50 (CAT)	31	1	2	28	11	2	3	3	3	4	2
E36 (ACC)	T48 (CAC)	26	2	2	22	4	3	4	2	2	5	2
E36 (ACC)	T49 (CAG)	17	2	0	15	3	1	4	1	1	1	4
E38 (ACT)	T47 (CAA)	39	0	1	38	5	6	11	5	2	3	6
E38 (ACT)	T50 (CAT)	38	0	4	34	0	8	12	8	0	5	1
E40 (AGC)	T50 (CAT)	40	1	5	34	4	4	4	10	1	8	3
E41 (AGG)	T47 (CAA)	41	4	4	33	6	6	5	5	5	3	3
E40 (AGC)	T49 (CAG)	35	5	4	26	8	6	6	0	0	5	1
E41 (AGG)	T50 (CAT)	30	2	2	26	5	3	4	4	4	1	5
E41 (AGG)	T59 (CTA)	20	3	3	14	0	1	3	1	1	4	4
Total number of markers		525	24	38	463	77	74	94	63	32	62	61

described by Jones et al. (1997), AFLP technology is very reliable and thus suitable for genetic mapping. The variation observed in DNA patterns may be due to the presence of *Tru*9I-*Tru*9I fragments which have escaped the kinetic enrichment for *Eco*RI-*Tru*9I fragments in the pre-amplification process (Vos et al. 1995) and have been detected by silver staining.

A total of 64 AFLP primer combinations with the selective bases at the 3′-end of each of the primers were tested on ten ryegrass plants in order to compare fingerprint patterns and determine which ones produced clearly detectable bands and revealed high levels of polymorphism. Of the 64 primer pairs tested, 17 were selected (E33T48, E33T50, E33T61, E33T62, E35T50, E35T59, E36T47, E36T48, E36T49, E36T50, E38T47, E38T50, E40T49, E40T50, E41T47, E41T50 and E41T59) for the generation and screening of DNA markers. Across these 17 primer combinations, the number of well-amplified bands varied between 60 and 120 while the number of polymorphic bands ranged from 17 (E36T49) to 41 (E41T47). Using a combination of both four- and six-base restriction enzymes, and both primers with three selective nucleotides, to detect polymorphism an average of 30 polymorphic markers per primer combination was found. This appears slightly higher compared to an average of 25 in hexaploid bread wheat (unpublished results) and 20 in rice (Maheswaran et al. 1997). Five-hundred and twenty five markers were scored on the 95 progeny using the 17 primer combinations shown in Table 1 and the data obtained was used for constructing the genetic map.

Data scoring

Segregation distortion is the deviation of segregation ratios from the expected Mendelian fraction and has been reported in a wide range of plant species (Jenczewski et al. 1997). Segregation distortion was observed for 86 of the 525 AFLP markers scored. Deviation from the expected ratio (1:1) was highly significant (P<0.001) for 24 markers initially placed in linkage group E. However these 24 markers were removed from further analysis in order to avoid false linkages. The remaining 62 skewed markers, demonstrating distorted segregation ratios (0.001<P<0.05), were retained for mapping. In total, 501 AFLP markers, were employed for the construction of the linkage groups. Of this total, 15 co-segregated with at least one other marker, 18 appeared to be allelic (although true allelism needs to be confirmed by sequence analysis) and 468 showed a unique segregation pattern.

The three isozyme markers (ACP/2, PGM and GOT/3) and the five EST markers (OSE, OSW, OSRB, LP1 and MZE) were scored as co-dominant and included in the linkage analysis.

Linkage analysis and map construction

Out of the 501 AFLP markers included in linkage analysis, 38 were unlinked. The 463 remaining AFLP markers were assigned to a framework of linkage groups at ∅= 0.25 and LOD=5. Seven linkage groups, presumably corresponding to the seven chromosomes in *Lolium* were formed with the 463 AFLPs, the five ESTs and the three isozyme markers. These seven linkage groups were consistent when using higher thresholds for LOD and ∅. Molecular markers were ordered within each linkage group at ∅=0.25 and LOD=5. The total map length generated was 930 cM. The average distance between adjacent markers was 1.97 cM. Linkage groups were assigned based upon their cM length (Fig. 1). The distribution of AFLP markers, generated with the *Eco*RI/Tru9I

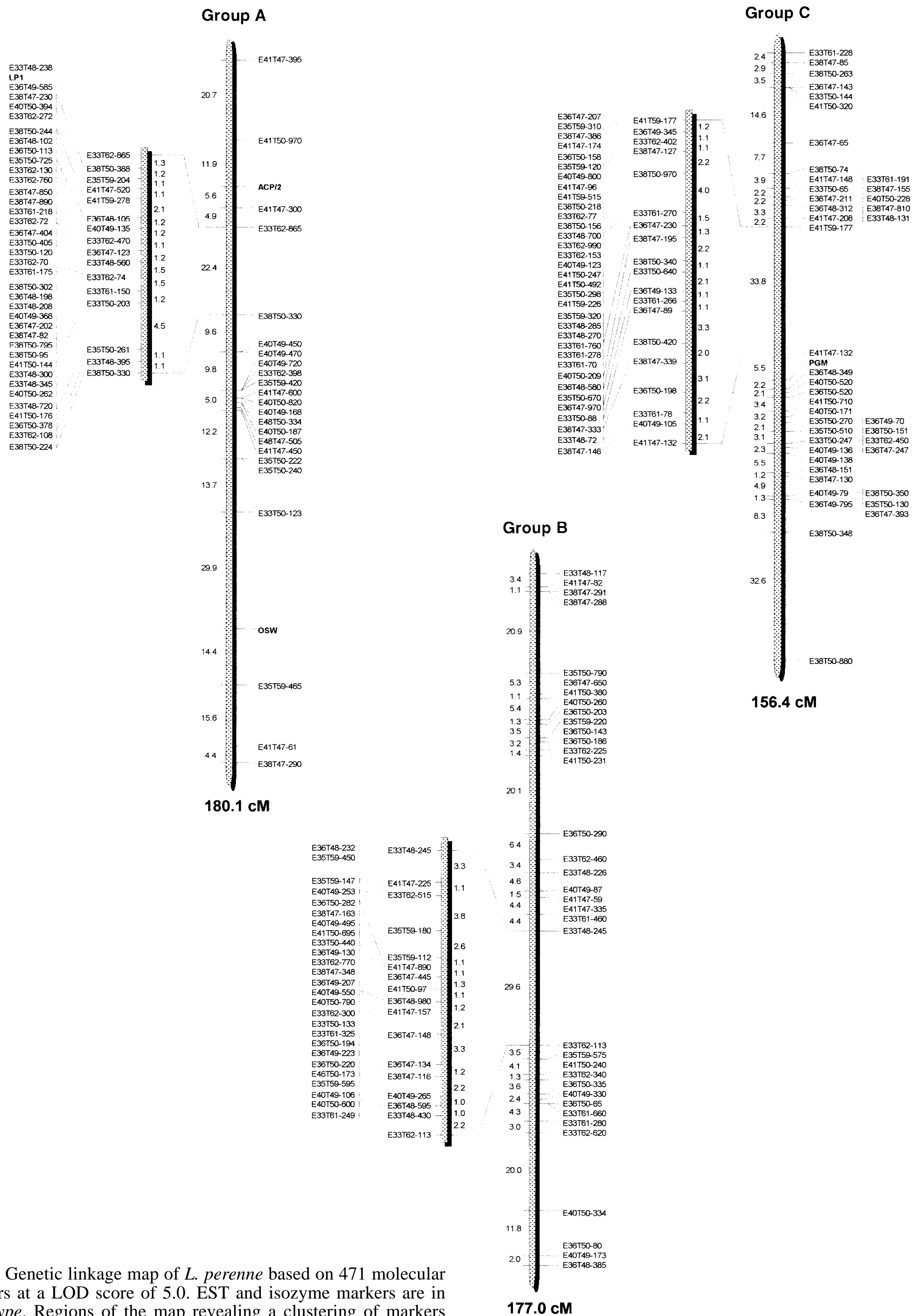

Fig. 1 Genetic linkage map of *L. perenne* based on 471 molecular markers at a LOD score of 5.0. EST and isozyme markers are in *bold type*. Regions of the map revealing a clustering of markers are shown expanded on the left. Genetic distances are in cM (Kosambi function)

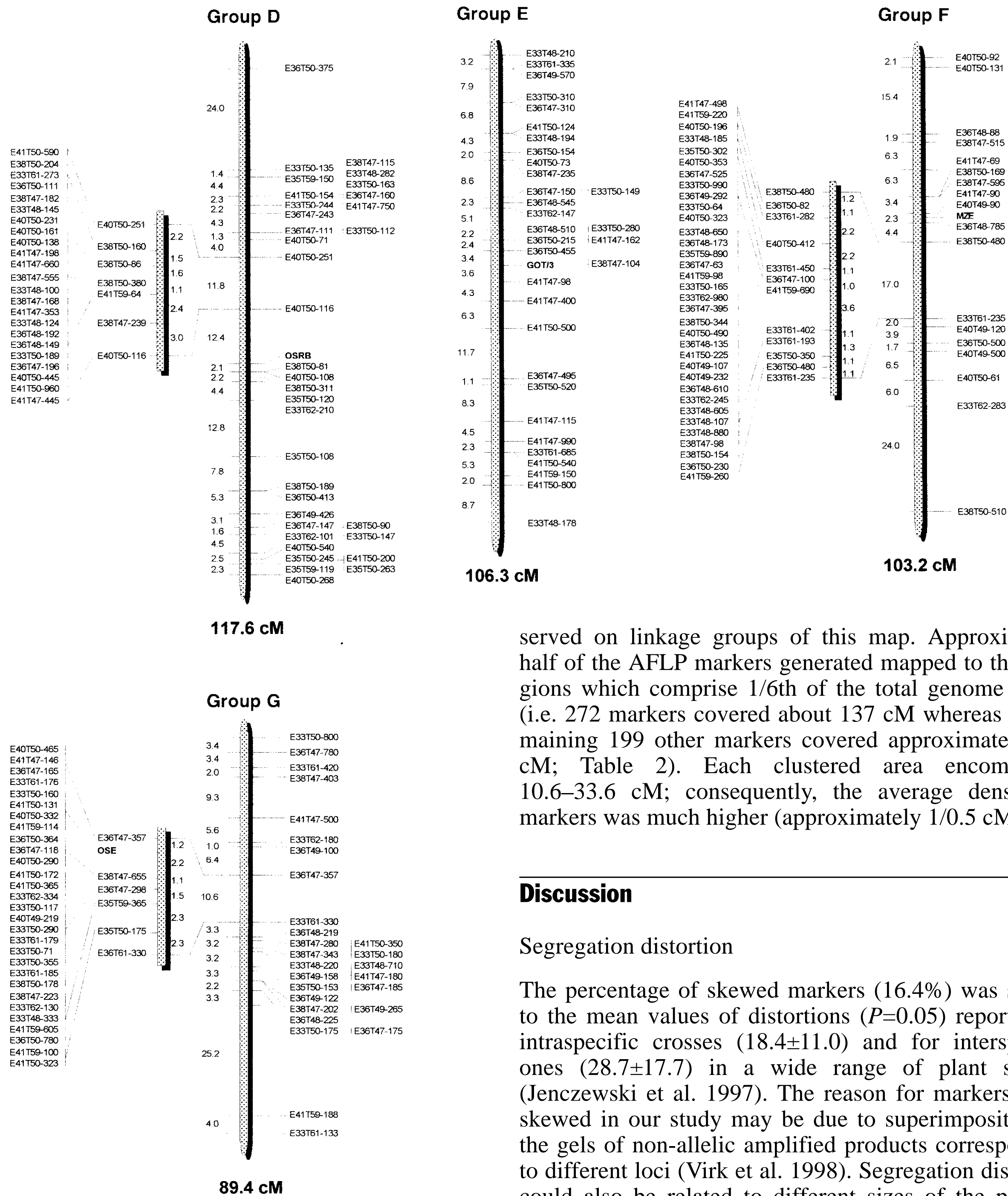

Fig. 1 (part II)

restriction enzymes, among the seven linkage groups was not proportional to the genetic lengths. The longest linkage group observed (linkage group A) is 180.1 cM and comprises 77 markers. In contrast, the shortest (linkage group G) is 89.4 cM and comprises 62 markers (Table 1). Most genetic distances between consecutive pairs of markers were smaller than 20 cM. Five gaps larger than 20 cM still exist on groups A, C, D, F and G.

The overall distribution within the linkage groups was not uniform. Strong clustering of AFLP markers was observed on linkage groups of this map. Approximately half of the AFLP markers generated mapped to these regions which comprise 1/6th of the total genome length (i.e. 272 markers covered about 137 cM whereas the remaining 199 other markers covered approximately 793 cM; Table 2). Each clustered area encompassed 10.6–33.6 cM; consequently, the average density of markers was much higher (approximately 1/0.5 cM).

Discussion

Segregation distortion

The percentage of skewed markers (16.4%) was similar to the mean values of distortions (P=0.05) reported for intraspecific crosses (18.4±11.0) and for interspecific ones (28.7±17.7) in a wide range of plant species (Jenczewski et al. 1997). The reason for markers being skewed in our study may be due to superimposition on the gels of non-allelic amplified products corresponding to different loci (Virk et al. 1998). Segregation distortion could also be related to different sizes of the parental genomes or to distorting factors such as self-incompatibility alleles. The 24 highly skewed markers initially all placed in group E were mapped to the vicinity of the isozyme marker GOT/3, previously designated as linked to the self-incompatibility Z locus (Hayward et al. 1998). Similar segregation distortion was observed for the GOT/3 locus by Hayward et al. (1998).

AFLP clustering

The clustering of AFLP markers observed in *Lolium* also appears with the *Eco*RI/*Tru*9I restriction enzymes in oth-

Table 2 Distribution of markers (463 AFLPs+5 EST+3 isoenzymes) among the seven linkage groups of ryegrass

Linkage group	Length (cM)	No. markers	Average distance (cM)	Clustering areas		Chromosome arms	
				Distance (cM)	No. markers	Distance (cM)	No. markers
A	180.1	77	2.34	22.4	54	157.7	23
B	177	77	2.30	29.6	43	147.4	34
C	156.4	95	1.65	33.6	53	122.8	42
D	117.6	64	1.84	11.8	30	105.8	34
E	106.3	33	3.22	12	9	94.3	24
F	103.2	63	1.64	17	47	86.2	16
G	89.4	62	1.44	10.6	36	78.8	26
Total	930	471	1.97	137	272	793	199

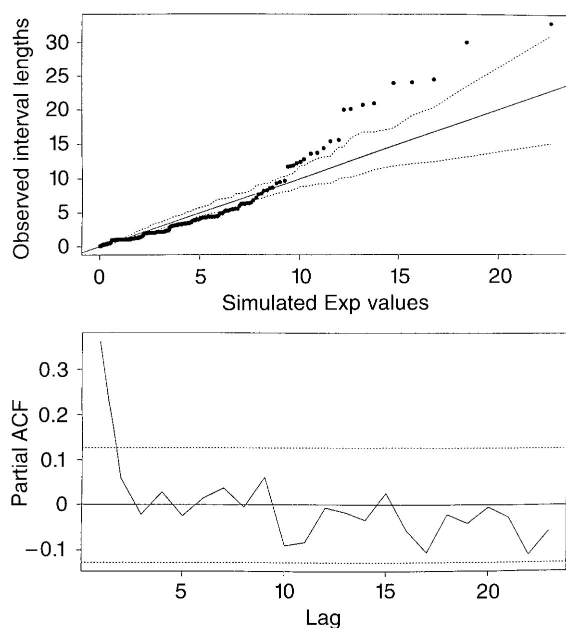

Fig. 2 Plot (upper part) of ordered observed distribution of interval lengths, against a series of 19 generated datasets from an exponential distribution using the same parameters as the observed one. The *thick line* is the first diagonal, *dotted lines* are the lowest and the highest simulated value, out of 19, corresponding to a rough 95% confidence interval; autocorrelation (lower part) of interval length values as a function of position. Dotted lines indicate the significance threshold at P=0.05

er plant AFLP linkage maps, such as *Arabidopsis* (Alonso-Blanco et al. 1998), barley (Qi et al. 1998), rice (Virk et al. 1998), and soybean (Keim et al. 1997) and may represent the centromeres. This suggest that the location of AFLP clusters in certain regions of the seven linkage groups might correspond to the centromeres of the *Lolium* chromosomes since the presence of heterochromatin in pericentromeric regions is a general feature of plant chromosomes. The centromeric suppression of recombination may be the mean reason for the clustering of markers (Tanksley et al. 1992) and the presence of repeated sequences in peri-centromeric areas (Alonso-Blanco et al. 1998; Qi et al. 1998). The randomness of marker distribution was tested in the following way: assuming that marker distribution is random, it may be assumed that the distribution of interval length follows an exponential law. Therefore, we simulated several sets of data from an exponential law with appropriate parameters in order to obtain expected values with their sampling distribution, and plotted observed against expected values. Figure 2 (upper part) clearly shows that observed values are mostly below expectation for small intervals, and above expectation for long ones. Thus, marker distribution is clearly not random. Clustering is equivalent to saying that small intervals are grouped together, which has been assessed by means of auto-correlation (Fig. 2, lower part). A significant positive value proves that intervals of a similar sizes (e.g. small ones) are clustered.

AFLP and genetic mapping

The AFLP technique will undoubtfully find many applications in high-density molecular mapping of both qualitative and quantitative trait loci (QTLs), and for marker-assisted selection (MAS). In order to ease the detection of AFLP markers in MAS or in genetic diversity studies, or to enhance the transferability of this kind of dominant locus specific marker (Alonso-Blanco et al. 1998; Qi et al. 1998) to other related populations, specific AFLP products can be transformed to a more-readily scored marker via cloning and sequencing to determine new primer sequences such as those designed for SCARs (sequence characterised amplified regions; Paran and Michelmore 1993). In this case, the application of silver staining for AFLP banding over other detection systems should prove to be a valuable tool by allowing the direct and simple recovery of DNA fragments from the gel.

The AFLP technique is a powerful tool for genetic mapping; however, it generates many redundant markers which tend to group into clusters. In order to reduced cost, it would be of interest to determine a subset of AFLP primers to be employed in order to achieve reasonable map coverage as a first genome framework. This can be done using a computerized optimization algorithm, the details of which will be presented in a subsequent paper.

We have successfully employed AFLP markers in the development of a high-density linkage map of *L. perenne* which compares well to those generated in other plants. A genetic linkage map based on molecular markers is a prerequisite for genetic studies and marker-assisted plant breeding. The current map based on 463 AFLP markers and eight other markers corresponding to expressed genes, extended to a total of 930 cM. The first *Lolium* map based on RFLP, RAPD and isozyme markers using an interspecific population was described by Hayward et al. (1994). The map established was 754 cM long (13 linkage groups), incorporating 61 markers. An extended map (Hayward et al. 1998) presented a total of 106 markers ascribed to seven linkage groups, covering a map distance of 692 cM with the allocation of two groups to chromosomes 2 and 6 of the *Lolium* genome.

The identification of linkage groups bearing the isozyme loci in the present study allows a consensus classification of the groups to be made along with those of the earlier investigations. Providing there has been no cytological changes such as translocations, group C carrying the PGM locus corresponds to group 5 and group E with the GOT/3 locus is concordant with group 2 of Hayward et al. (1998). The map lengths established for group E in the present study is close to that identified previously for group 2 (106 to 100 cM) whilst for group C compared with group 5 it has been extended (156 compared to 97 cM). Further analysis using common RFLPs or other markers will allow full concordance to be established between the maps.

We can reasonably suppose that the total length of the ryegrass genome is not yet completely covered since the 38 unlinked markers might extend chromosome arms. The genetic map presented here could be extended using other restriction enzymes (such as the *Pst*I-*Mse*I combination) for AFLP markers, or by using RFLP and microsatellite markers from other related species. This would allow gaps between distant markers to be filled as well as an assessment of the homoeologous relationship between *Lolium* chromosomes with those of the *Triticeae*.

Future prospects in *Lolium* mapping

It is to be expected that the p150/112 population used for the construction of our AFLP map will form a useful reference population to identify loci involved in important developmental and physiological traits, as recently proposed by the formation of the International *Lolium* Genome Initiative (ILGI; Forster 1999).

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References

Adams MD, Kelley JM, Gocayne JD, Dubnick M, Polymeropoulos MH, Xiao H, Merril CR, Wu A, Olde B, Moreno F, Kerlavage AR, McCombie RW, Venter JC (1991) Complementary DNA sequencing: expressed sequence tags and the human genome project. Science 252:1651–1656

Alonso-Blanco C, Peeters AJM, Koornneef M, Lister C, Dean C, van den Bosch N, Pot J, Kuiper TR (1998) Development of an AFLP-based linkage map of L, Col and Cvi *Arabidopsis thaliana* ecotypes and construction of a L/Cvi recombinant inbred line population. Plant Jour 14:259–271

Botstein D, White RL, Skolnik M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Caetano-Anolles G, Bassam BJ, Gresshoff PM (1991) DNA fingerprinting using very short arbitrary oligonucleotides. Bio/technology 9:553–557

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group-7 chromosomes. Theor Appl Genet 78:495–504

Devos K, Gale M (1993) The genetic maps of wheat and their potential in plant breeding. Outlook Agric 22:93–99

Forster JW (1999) The International *Lolium* Genome Initiative. In: Plant and Animal Genome VIIth Conf, San Diego, California, January 17–21, 1999, pp 53

Hayward MD, McAdam NJ, Jones JG, Evans C, Evans GM, Forster JW, Ustin A, Hossain K G, Quader B, Stammers M, Will JK (1994) Genetic markers and the selection of quantitative traits in forage grasses. Euphytica 77:269–275

Hayward MD, Degenaars GH, Balfourier F, Eickmeyer F (1995) Isozyme procedures for the characterization of germplasm, exemplified by the collection of *Lolium perenne* L. Genet Resour Crop Evol 42:327–337

Hayward MD, Forster JW, Jones JG, Dolstra O, Evans C, McAdam NJ, Hossain KG, Stammers M, Will J, Humphreys MO, Evans GM (1998) Genetic analysis of *Lolium*. I. Identification of linkage groups and the establishment of a genetic map. Plant Breed 117:451–455

Hutchinson J, Rees H, Seal AG (1979) An assay on the activity of supplementary DNA in *Lolium* . Heredity 43:411–421

Jenczewski E, Ghererdi M, Bonnin I, Prosperi J M, Olivieri I, Huguet T (1997) Insight on segregation distortion in two intraspecific crosses between annual species of Medicago (Leguminosae). Theor Appl Genet 94:682–691

Jones CJ, Edwards KJ, Castaglione S, Winfiefd MO, Sala F, van de Wiel C, Bredemeijer G, Vosman B, Matthes M, Daly A, Brettschneider R, Bettini P, Buiatti M, Maestri E, Malcevschi N, Aert R, Volckaert G, Rueda J, Linacero R, Vazquez A, Karp A (1997) Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Mol Breed 3:381–390

Karp A, Seberg O, Buiatti M (1996) Molecular techniques in the assessment of botanical diversity. Ann Bot 78:143–149

Keim P, Schupp JM, Travis SE, Clayton K, Zhu-T, Shi LA, Ferreira A, Webb DM (1997) A high-density soybean genetic map based on AFLP markers. Crop Sci 37:537–543

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lallemand J, Lem P, Ghesquiere M, Charmet G, Balfourier F (1998) Potentiality of STS for variety distinction in ryegrass. In: Working Group on biochemical and molecular Techniques and DNA-profiling in particular. 5 Session, Betsville, USA, September 28–30, 1998, UPOV (Ed.), BMT/S/11

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary linkage maps of experimental and natural populations. Genomics 1:174–181

Litt M, Luty J (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44:391–401

Maheswaran M, Subudhi PK, Nandi S, Xu JC, Parco A, Yang DC, Huang N (1997) Polymorphism, distribution, and segregation of AFLP markers in a doubled-haploid rice population. Theor Appl Genet 94:39–45

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985–993

Qi X, Stam P, Lindhout P (1998) Use of locus-specific AFLP markers to construct a high-density molecular map in barley. Theor Appl Genet 96:376–384

Roder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Rogers SO, Bendich AJ (1985) Extraction of DNA from milligram amounts of fresh herbarium and mummified plant tissues. Plant Mol Biol 5:69–76

Saiki RK, Gelfland DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Ehrlich HA (1988) Primer-directed enzymatic amplification of DNA fragment length polymorphism loci in rice. Jpn J Breed 41:665–670

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant Jour 3: 739–744

Tanksley SD, Ganal MW, Prince JP, De Vincente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller L, Paterson AH, Pineda O, RMS, Wing RA, Wu W, Young ND (1992) High-density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Terrell EE (1968) A taxonomic revision of the genus *Lolium*. Tech Bull US Dept Agric 1392:1–65

Tixier MH, Sourdille, RM, Leroy P, Bernard M (1997) Detection of wheat microsatellites using a non radioactive silver-nitrate staining method. J Genet Breed 51:175–177

Virk PS, Ford-Lloyd BV, Newbury HJ (1998) Mapping AFLP markers associated with subspecific differentition of *Oryza sativa* (rice) and an investigation of segregation distortion. Heredity 81:613–620

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 19:861–866

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6531–6535

Zabeau M, Vos P (1993) Selective restriction fragment amplification: a general method for fingerprinting. European Patent Application 924026 29.7 (publ. no.: 0534858 A1)