Theor Appl Genet (2002) 104:273–280

A. Porceddu · E. Albertini · G. Barcaccia
E. Falistocco · M. Falcinelli

Linkage mapping in apomictic and sexual Kentucky bluegrass (*Poa pratensis* L.) genotypes using a two way pseudo-testcross strategy based on AFLP and SAMPL markers

Received: 31 August 2000 / Accepted: 12 January 2001

Abstract The high versatility of the mode of reproduction and the retention of a pollen recognition system are the factors responsible for the extreme complexity of the genome in *Poa pratensis* L. Two genetic maps, one of an apomictic and one of a sexual genotype, were constructed using a two-way pseudo-testcross strategy and multiplex PCR-based molecular markers (AFLP and SAMPL). Due to the high ploidy level and the uncertainty of chromosome pairing-behavior at meiosis, only parent-specific single-dose markers (SDMs) that segregated 1:1 in an F_1 mapping population (161 out of 299 SAMPLs, and 70 out of 275 AFLPs) were used for linkage analysis. A total of 41 paternal (33 SAMPLs and 8 AFLPs) and 47 maternal (33 SAMPLs and 14 AFLPs) SDMs, tested to be linked in coupling phase, were mapped to 7+7 linkage groups covering 367 and 338.4 cM, respectively. The comparison between the two marker systems revealed that SAMPL markers were statistically more efficient than AFLP ones in detecting parent-specific SDMs (75% vs 32.4%). There were no significant differences in the percentages of distorted marker alleles detected by the two marker systems (27.8% of SAMPLs vs 21.3% of AFLPs). The pairwise comparison of co-segregational groups for linkage detection between marker loci suggested that at least some of the *P. pratensis* chromosomes pair preferentially at meiosis-I.

Communicated by F. Salamini

E. Albertini · E. Falistoco · M. Falcinelli (✉)
Dipartimento di Biologia Vegetale
e Biotecnologie Agroambientali. University of Perugia,
Borgo XX Giugno 74, 06121, Perugia, Italy

G. Barcaccia
Dipartimento di Agronomia Ambientale e Produzioni Vegetali,
University of Padova, Agripolis, Via Romea 16, 35020 Legnaro,
Padova, Italy

Current address:
A. Porceddu, CNR-IMOF, Viale dell' Università 133,
80055 Portici Napoli, Italy

Both A. Porceddu and E. Albertini contributed equally to this manuscript

Keywords AFLP · Mapping · Polyploids · *P. pratensis* · SAMPL

Introduction

Kentucky bluegrass (*Poa pratensis* L.) is a hardy, persistent forage and turf grass adapted to a wide range of soils and climates (Meyer and Funk 1989). Its ever-increasing adoption in highly cared-for sports fields (e.g. soccer, rugby, golf) has attracted the attention of many seed companies. However, in the past, the breeding of superior varieties was often hampered by the extreme complexity of the genome (Bashaw and Funk 1987). Several factors are responsible for such complexity.

Firstly, the marked versatility of the mode of reproduction, which in *P. pratensis* ranges from nearly obligate apomixis to complete sexuality, permits the formation and perpetuation of highly polyploid or even aneuploid genotypes. The variable chromosome number is the result of either mitotic aberrations, that occur in both the vegetative and reproductive meristems, or cytological abnormalities during male and female sporogenesis (D'Amato 1995).

Secondly, the combination of a pollen recognition system and the apomeiotic nature of apomixis confers a strong ability to hybridize and retain alien genomes (Wedin and Huff 1996), so determining high ploidy levels (x=7, 2n=28–147, Speckmann and van Dick 1972) and unusual chromosome numbers ($2n$=36–123, Darlington and Wylie 1961), which further complicates genetic analysis.

Biochemical (Glaszman et al. 1989) and molecular (D'Hont et al. 1994) markers are helpful for studying unifactorial inheritance in such complex genomes (Glaszman et al. 1997). RFLP markers have, for example, been employed for linkage-map construction and genome-organization studies in highly polyploid species such as cultivated sugarcane (Grivet 1996). Although this molecular-marker system has the potential to screen several markers, or alleles, simultaneously, due to limited polymorphisms it is often unable to identify as

many distinct alleles for a given locus as the ploidy level could allow. Further complexity can be caused by sequence duplications in the genome, owing to the presence of paralogous loci.

Multilocus PCR-based molecular markers, such as AFLPs (Zabeau and Vos 1992) and SAMPLs (Morgante and Vogel 1994), have proved very useful for developing and integrating genetic maps of many dicot and monocot species. The AFLP marker system provides a molecular assay that combines the reliability of the RFLPs with the power of the PCR. The SAMPL analysis extends AFLP technology and enables the amplification of microsatellite regions without prior cloning and characterization of specific microsatellite loci. The key to the success of these markers is to be found in their high multiplex ratio, which allows many loci to be checked simultaneously. However, the extreme complexity of the banding profiles visualized makes identifying the marker alleles, and in consequence performing some genetic analyses, far from easy. Additional complications may arise in some polyploid species owing to the uncertainty of the genome constitution (allopolyploids vs autopolyploids), thereby making it difficult to determine the pattern of inheritance. Species with high ploidy levels may originate through both autopolyploidy and allopolyploidy (Averett 1980). A practical way to handle the molecular marker data sets in these species is to treat each band as a marker allele for a single locus of a given genotype and to infer its allelic dose by studying the progeny segregation ratio (Wu et al. 1992; Hackett et al. 1998; Ripol et al. 1999). A band present in a single dose (hereafter defined as SDM=single-dose marker) in a heterozygous plant, for example, will be inherited in half of the gametes. If this plant is selfed the progeny would be expected to have a segregation ratio of 3:1 (presence vs absence). The situation becomes more intricate when the segregation ratio of two SDMs has to be deciphered. Two SDMs may be either linked in coupling on the same chromosome, linked in repulsion in two homologous chromosome or completely unlinked. Both the expected segregation ratios and the linkage equations for SDMs linked in coupling are equivalent to those for diploids (Wu et al. 1992). The same holds for repulsion-phase linkages in species with a preferential pairing (allopolyploids, Wu et al. 1992). The definition of the expected segregation ratios is more complicated in autopolyploids and relies on many factors, including the ploidy level, the chromosome-pairing behavior and the extent of double reduction. Moreover, as shown by Wu et al. (1992), the power of detection of repulsion-phase linkages in autopolyploids is acceptable only with numerous progeny plants. For example, with a population size of 67 and a confidence level of 5%, the maximum detectable distance in autotetraploids is 7.4 cM and no linkages can be detected at higher ploidy levels.

Early cytological observations demonstrated that the pairing behavior of *P. pratensis* is mostly as bivalents with a low percentage of multivalents (Love and Love 1975). Nothing is known about its genomic constitution (autopolyploidy vs allopolyploidy).

We constructed two linkage maps, one of an apomictic, the other of a sexual, genotype, based on a two-way pseudo-testcross strategy. Only single-dose AFLP and SAMPL markers polymorphic between parents, that were verified to segregate 1:1 in the F_1 mapping population, were used in linkage analysis. Pairing of the chromosomes of *P. pratensis* is also discussed.

Material and methods

Plant material and controlled matings

A segregating F_1 population of 67 plants was produced by crossing a completely sexual clone (S1/1–7) with a highly apomictic genotype (RS7–3). The sexual clone derived from a cross between two completely sexual genotypes selected from German cultivars (Matzk 1991; Barcaccia et al. 1998), while the apomictic clone came from an Italian natural population (Mazzucato 1995).

Cytological investigations

For chromosome counts, the analysis of metaphase plates was performed in the root tips of parents and of 20 randomly chosen progeny plants. Excised root tips were placed in ice cold water for 26–28 h, then in a saturated solution of α-bromonaphthalene for 3 h and finally fixed in ethanol:acetic acid (3:1). After Feulgen staining, squashes were prepared in 1.5% (w/v) acetic orcein and attached to a cover slip with glycerin-albumin. The chromosome number was determined in 10 to 15 metaphases for each genotype, under an optical photo-microscope (Nikon).

DNA isolation and markers analysis

Genomic DNA of single F_1 plants and parents was extracted using the CTAB procedure (Doyle and Doyle 1990). AFLP marker analysis was carried out according to Vos et al. (1995) as modified by Cnops et al. (1996). A fluorescent-labelled EcoRI+CNN primer was used for selective PCRs.

SAMPL marker analysis was performed according to Morgante and Vogel (1994). The primer used for the selective amplifications was the fluorescent-labelled AS1 (CACACACACACACACTATAT). The list of primer combinations is reported in Table 1.

After PCR, for both AFLP and SAMPL, 8 µl of loading buffer (98% formamide, 2% dextran blue, 0.25 mM EDTA) were added to each tube. Samples were denatured at 90°C for 5 min and then immediately placed on ice. For each sample 6 µl were loaded onto a 6% polyacrylamide gel (60 cm×30 cm×0.4 mm), which had been run for 2 h and 45 min at 80 W. Gels were scanned using the Genomyx LR scanner (Beckman Coulter Corporation, Calif.).

Table 1 List of *Eco*RI/*Mse*I, and SAMPL/*Mse*I primer combinations indicated as the combination of the three selective nucleotides of each primer

AFLP *Eco*RI+3/*Mse*I+3 primers		
CCA/AAA	CCA/ACG	CAA/AAC
CCA/AAC	CCA/AAT	CAA/AAT
CCA/ACA	CCA/AGA	CAA/AAG
SAMPL AS1/ MseI+3 primers		
AAT	ACA	AGA
AGT	ACT	AGC
AGG	ACG	AAC
AAG	ATA	ACC

Segregation analysis

In species with complex genomes, such as *P. pratensis*, it is very important to distinguish SDMs from non-SDMs. Only SDMs, i.e. markers due to a single allele of a given locus, were used for linkage-mapping analysis. A segregation ratio of 2.45 giving an equivalent χ^2 for both the 1:1 and 3:1 hypotheses was adopted as border line of SDM. Only markers polymorphic between parents and with a segregation ratio lower than 2.45 were considered as SDM. The segregation patterns (presence vs absence) of marker alleles observed in the F_1 mapping population were assayed by χ^2 analysis to test the goodness of fit with the 1:1 ratio expected for parental loci having a simplex by nulliplex condition.

The observed numbers of SAMPL and AFLP parental polymorphisms, segregating parental SDMs, and mapped marker loci were analyzed by 2×2 contingency tests in order to compare the efficiency of the two molecular-marker systems.

Linkage mapping

The F_1 progeny plants were scored for marker polymorphisms. The observed segregation ratio of AFLP and SAMPL markers was tested by chi-square analyses for goodness of fit to the expected segregation ratios, as well as by the Mather test (Mather 1957) to assess the phase of linkage. Marker-segregation data were analyzed with JOINMAP Version 2.0 (Stam and Van Ooijen 1995) by treating the segregation data of markers as a "backcross" ("BC_1") population. The function "group" was applied by setting a LOD score of 2.5 and a maximum recombination frequency r=0.35 to detect groups of linked markers. Data were also analyzed by using the "cross pollination" ("CP") population-type option, i.e. an F_1 population resulting from a cross between two heterogeneous parents, which were respectively simplex and nulliplex at the loci being tested. For the identification of linkage groups, the "group" module was applied by setting a minimum LOD score of 2.5 and a maximum recombination frequency r=0.35. The "splitting" module was employed for both mapping methods to order marker loci within each linkage group. The parameter "ripple" was applied to improve the final order of the marker loci. Map distances, expressed in centiMorgans (cM), were calculated by the Kosambi function (Kosambi 1944).

Results

Cytological investigation

Cytological investigations showed that the chromosome number of S1/1–7 and RS7–3 were 2n=36 and 2n=64, respectively (Fig. 1a, b). All 20 progeny plants investigated had a 50-chromosome complement (Fig. 1c) and their karyotype was mainly constituted of metacentric chromosomes, which, however, had different sizes. The larger pairs were on average 2-fold greater than the smaller ones. The differential morphological characteristics, potentially useful for investigating the genomic organization, could not be visualized.

Segregation analysis

Markers segregating from RS7–3, the apomictic parent, were nominated as "paternal", those segregating from S1/1–7, the sexual parent, "maternal", those segregating from both parents as "shared".

A total of 275 AFLP polymorphisms were detected in the F_1 progeny using nine primer combinations with an average of 30.5 polymorphisms per primer combination; the maximum was 43 polymorphic fragments with the primer combination *Eco*+CCA/*Mse*+AAT and the minimum 11 with the primer combination *Eco*+CAA/*Mse*+AAA. Sixty polymorphisms (21.8% of the total) were maternal, 65 paternal (23.7%), and the remaining 150 were shared (54.5%).

A total of 299 SAMPL polymorphisms were detected using 12 primer combinations, with an average of 24.9

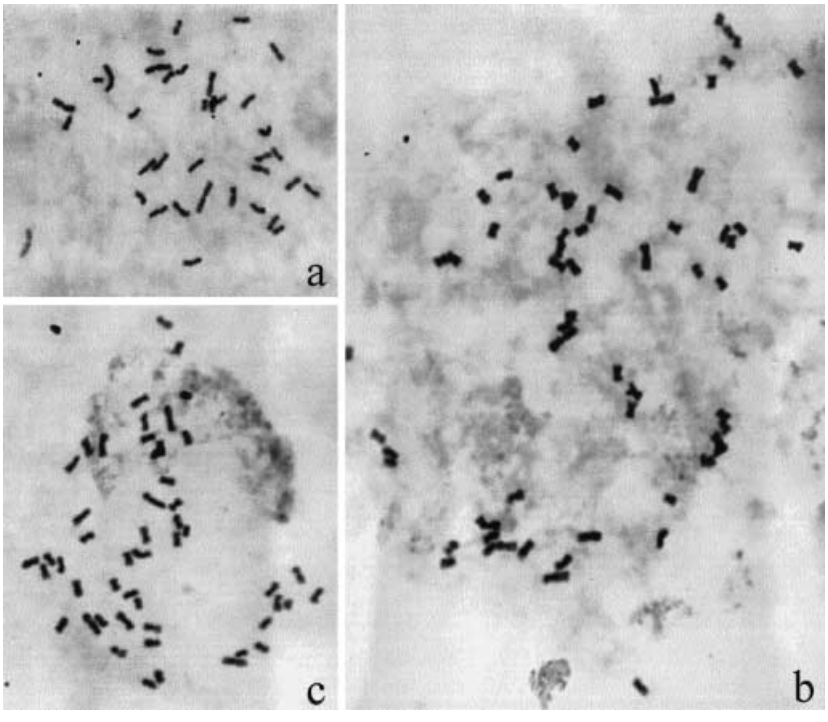

Fig. 1a–c Somatic metaphases of *P. pratensis*. **a** maternal plant (S1/1–7) with 2n=36; **b** paternal plant (RS7–3) with 2n=64; and **c** one of the progeny plants with 2n=50

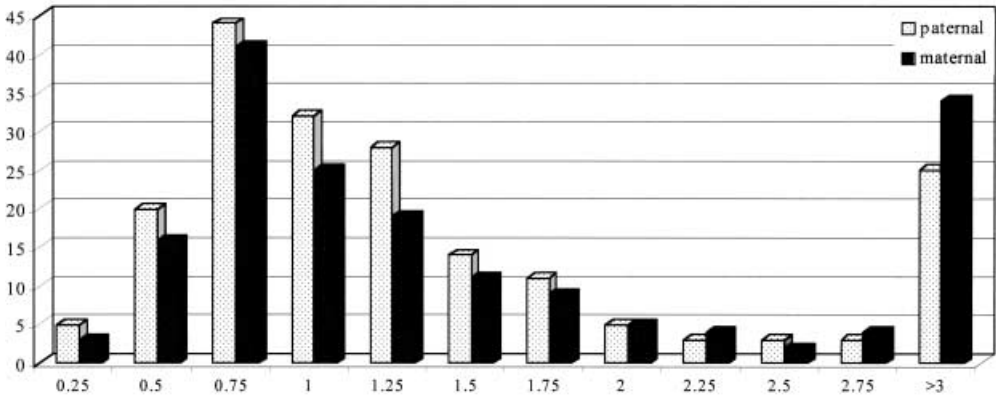

Fig. 2 Distribution of segregation ratios (presence: absence) of the 193 paternal and 173 maternal markers polymorphic between parents revealed by 9 AFLP and 12 SAMPL primer combinations in the F_1 progeny. Markers with a ratio greater than 2.5:1 were considered to represent other than SDMs and were not used for mapping

Table 2 Information on the accord between the observed and expected segregation patterns for single-dose maternal and paternal marker alleles

Marker type	Marker origin	No. of marker alleles	χ^2 ($P\geq0.05$) mean±SE	No. of marker alleles	χ^2 ($0.05>P\geq0.01$) mean±SE	No. of marker alleles	χ^2 ($P<0.01$) mean±SE
AFLP	Paternal	30 (75.0%)	0.890±0.171	7 (17.5%)	6.149±0.423	3 (7.5%)	7.896±0.711
	Maternal	40 (81.6%)	0.708±0.133	5 (10.2%)	5.735±0.485	4 (8.2%)	7.106±0.537
SAMPL	Paternal	83 (70.9%)	1.015±0.131	15 (12.8%)	5.189±0.196	19 (16.3%)	15.641±3.210
	Maternal	78 (73.6%)	1.191±0.113	16 (15.1%)	4.964±0.234	12 (11.3%)	12.024±1.807
Overall paternal alleles		113 (72.0%)	0.982±0.164	22 (14.0%)	5.494±0.322	22 (14.0%)	14.585±3.443
Overall maternal alleles		118 (76.1%)	1.027±0.158	21 (13.6%)	5.148±0.353	16 (10.3%)	10.795±1.490

polymorphisms per primer combination; the maximum was 44 with the primer combination AS1/*Mse*+ACA and the minimum eight with the primer combination AS1/*Mse*+ACC. Of these, 113 were maternal (37.8%), 128 paternal (42.8%) and 58 shared (19.4%).

Chi-square analyses for various segregation ratios (from 1:4 up to 4:1, presence vs absence) were performed. Figure 2 gives the distribution of segregation ratios for the maternal and paternal marker alleles. Both distributions were skewed toward low values, which correspond to a marker allele presence lower than expected. The most-represented segregation ratio was 0.75 for both, with mean ratios of 1.06 for S1/1–7 and 1.07 for RS7–3 markers.

Comparison of the AFLP and SAMPL markers in detecting parent-specific SDMs in *Poa pratensis*

Of the SAMPL polymorphisms, 75% (223) were inherited as parent-specific SDMs (117 paternal and 106 maternal). This corresponds, on average, to 18.6 (9.75 paternal and 8.85 maternal) SDMs per primer combination. In contrast, only 32.4% (89) of the AFLP polymorphisms showed segregation ratios attributable to a parent-specific SDM (40 paternal and 49 maternal), with an average of 9.9 (4.45 paternal and 5.45 maternal) SDMs per primer combination. However, there were no significant differences in the relative contribution of the two types of molecular markers to the various SDM segregation classes (data not shown). On the whole, the mean number of parent-specific SDM polymorphisms supplied per assay by the two marker systems (18.5 SAMPLs vs 9.8 AFLPs) differed significantly ($\chi^2=28.11$, $P<0.01$).

Overall, 113 paternal (30 AFLPs and 83 SAMPLs) and 118 maternal (40 AFLPs and 78 SAMPLs) SDMs segregated in a 1:1 Mendelian fashion ($P\geq0.05$; Table 2). Of the remaining 44 paternal (10 AFLPs and 34 SAMPLs) and 37 maternal (9 AFLPs and 28 SAMPLs), only 22 paternal (3 AFLPs and 19 SAMPLs) and 16 maternal (4 AFLPs and 12 SAMPLs) SDMs were markedly distorted ($P<0.01$; Table 2). In conclusion, segregation distortion of SAMPL SDMs (62 out of 223, 27.8%) did not differ significantly ($\chi^2=1.063$, $df=1$) from that of the AFLP SDMs (19 out of 89, 21.3%).

Linkage mapping

Cytological studies have shown that pairing behavior is mostly as bivalents in *P. pratensis*, with small percentages of trivalents and tetravalents. However, it is not known whether the bivalents are formed randomly or preferentially. Because linkage detection in the repulsion phase in autopolyploids requires large numbers of progeny plants (see Wu et al. 1992), segregation sets are usually limited to SDMs linked in the coupling phase. Only SDMs showing a 1:1 segregation ratio (for $P\geq0.05$) were used for mapping.

Forty one of the 113 paternal SDMs (33 SAMPLs and 8 AFLPs) were mapped to seven linkage groups (Fig. 3a) covering 367 cM (on average 52.4 cM per

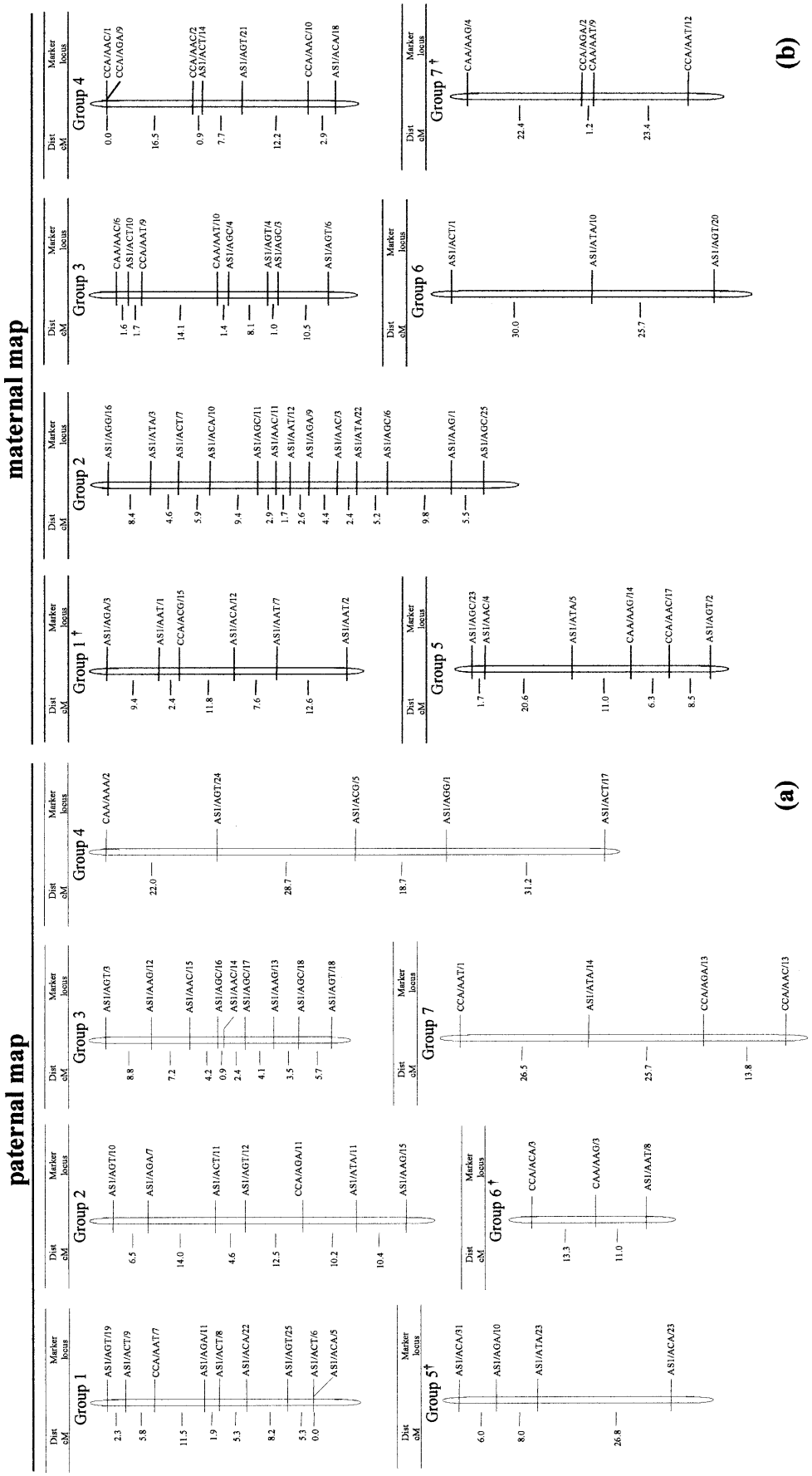

Fig. 3 Genomic linkage map obtained using the BC_1 mapping population option of **a** the paternal genotype and **b** the maternal genotype. Distances are expressed in cM and were calculated using the Kosambi function. AFLP locus designations refer to the *Eco*RI/*Mse*I selective combination of bases, whereas those of SAMPL refer to the *Mse*I selective combinations with the AS1 primer. †Co-segregational groups that showed a non-significant 1:1 χ^2 test (for $P \leq 0.05$) suggesting a preferential chromosome pairing behavior

group and 5.8 markers per group). Forty seven of the 118 maternal SDMs (33 SAMPLs and 14 AFLPs) were mapped to seven linkage groups (Fig. 3b) covering 338.4 cM (on average 48.3 cM per group and 6.7 markers per group).

Additional SDMs (19 paternal and 18 maternal) were found to belong to the linkage groups detected, but they have not been included in the two maps because the "ripple" function was unable to confirm the final order of marker loci.

The paternal map included 39.7% and 26.6% of the paternal SAMPL and AFLP SDMs, while the maternal map included 42.3% and 35% of the maternal SAMPL and AFLP SDMs. On the whole, 36.2% of the paternal SDMs segregating 1:1 and 39.8% of the maternal ones were mapped. There was a significant difference (χ^2=5.96; $P \leq 0.05$) between the proportion of SAMPL and AFLP marker alleles mapped as segregating 1:1 in the F_1 mapping population (i.e. 66 out of 161, 40.9%, and 22 out of 70, 31.4%).

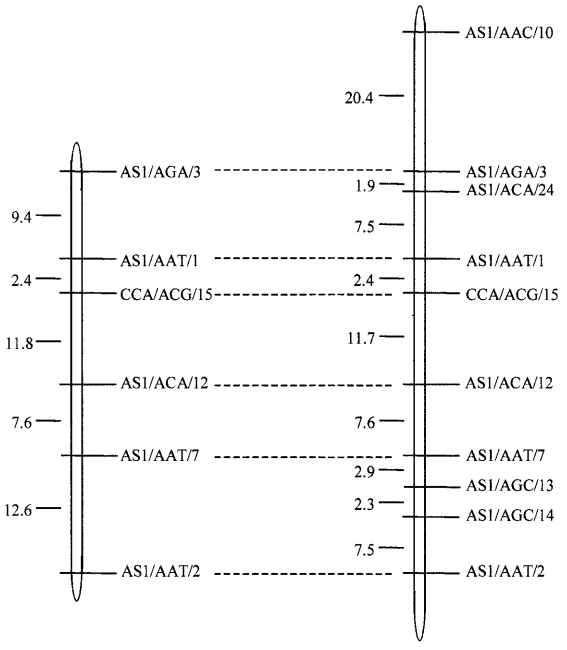

Fig. 4 Comparison of maternal linkage group 1 as obtained with the BC_1 **(a)** and CP **(b)** mapping population options

Discussion

There are several reasons why the construction of genetic maps of polyploid species has long lagged behind that of diploid species: (1) the statistical methods are far more-complicated than for diploids; (2) large segregating populations are needed to obtain reliable genetic distance estimates; and (3) little is known about the genomic constitution of most polyploids. The genetic maps constructed for some polyploid species, e.g. alfalfa (Kiss et al. 1993) and potato (Jacobs et al. 1995), have been based on closely related diploid species, but it would be impossible and unwise to apply such a strategy to species that have no close diploid relatives or whose genomic constitution is poorly defined. *P. pratensis* is a species whose chromosome number varies from a minimum of 28 to a maximum of 147. The reasons for the extended chromosome number variation are to be sought in the high frequency of mitotic and meiotic aberrations and the marked degree of hybridization with other species (Clausen 1961).

Because an intercross between sexual and apomictic genotypes is almost obligatory for introgressing a desired trait into an apomictic genetic background, we performed a first genetic map of *P. pratensis* using an F_1 population of 67 individuals obtained from a cross between a sexual and an apomictic parent. This strategy yielded two maps, one for the apomictic and the other for the sexual parent.

Cytological studies revealed that the chromosome number was uniform in all the F_1 plants, thus indicating that, even though the chromosome number of the parents was not a multiple of 7 (2n=36 and 2n=64), the meiotic

Chromosome pairing behavior in *P. pratensis*

Wu et al. (1992) have reported that, when chromosomes pair preferentially, the power of detection of repulsion- and coupling-phase linkages coincides. As a consequence, if in a given pair of cosegregating groups, the number of markers in coupling is comparable to the number of markers in repulsion the chromosomes would pair preferentially.

Performing the Mather test (Mather 1957), maternal and paternal SDMs were ordered in 35 co-segregational groups. SDMs of each pair of co-segregational groups were then tested for linkages in repulsion. The ratios of SDMs linked in coupling versus those linked in repulsion calculated for each pairwise comparison was then assayed for a 1:1 fit (Wu et al. 1992). Two types of situations were distinguished: (1) paternal groups 5 and 6 and maternal groups 1 and 7 showed a non-significant value (at $P \leq 0.05$) suggesting the hypothesis of preferential pairing behavior and repulsions between markers were strong (LOD > 3); (2) Repulsion involved small co-segregating groups of two or three markers and it was difficult to further investigate the distribution of repulsion between groups.

To further test the hypothesis, a linkage analysis was performed using the CP mapping-population option which allows markers in both the coupling and repulsion phases to be linked as in diploid and allopolyploid species. Additional markers were mapped only in those groups indicated by the Mather test as being interested by preferential chromosomes pairing. These additional alleles were mapped in repulsion alongside a framework of marker alleles in coupling, without changing either the distances or the order of coupling markers previously identified with the BC_1 mapping population option. Figure 4 gives an example of a linkage group obtained by BC_1 and CP options.

segregation was regular (18:18 for the 2n=36 female parent and 32:32 for the 2n=64 male parent) and produced a progeny with a uniform chromosome complement of 2n=50. Being x=7, the complement of the sexual parent could be composed by four complete sets plus four couples of "additional" chromosomes, whereas the complement of the apomictic parent could be of eight complete sets plus four couples of "additional" chromosomes. Extended studies are needed to ascertain the origin and number of these additional chromosomes.

A crucial step in constructing genetic maps in polyploid species is the determination of marker dosage. This is usually done by counting the proportion of plants in the progeny that exhibit the marker, and testing this proportion against those expected for various marker dosages.

A molecular marker segregating from one parent may be present as a single allele (single-dose marker=SDM), two alleles (double-dose marker=DDM), three alleles (TDM), etc. Whereas SDMs are expected to segregate in the same way regardless of the ploidy level and genome constitution, the expected segregation ratio of higher-dose markers such as DDM, TDM, etc., varies according to the genome constitution (autopolyploidy vs allopolyploidy), the ploidy level, and the pairing behavior (bivalents, trivalents, etc.). A DDM of an allopolyploid, for example, may be inherited in all or three-fourths of the gametes, depending on whether the two copies of the marker allele are on homologous or homoeologous chromosomes. The expected segregation ratio (presence vs absence) in gametes of autopolyploids with bivalent pairing at meiosis is, instead, (3h-2):(h-2) where h represents the ploidy level. At high ploidy levels the segregation ratio approaches 3:1 (presence vs absence) (Sorrels 1992).

Of the 366 molecular markers polymorphic between parents, 312 (85.2%) were inherited as SDMs. Interestingly, the SAMPL technique was more efficient in detecting SDMs than the AFLP procedure. The higher number of sources responsible for SAMPL polymorphism, and/or a different genomic distribution of the two types of molecular markers, probably explain this finding.

An F_1 population obtained by crossing two highly heterozygous parents, characterized for their different reproductive behavior, was studied assuming that a non-inbred population would not only provide an effective strategy for limiting segregation distortion, but also give better estimates of linkage distances (Tavoletti et al. 1996). In fact, 75% of SDMs (231 out of 312) segregated according to a Mendelian ratio of 1:1, and only 25% of SDMs (81 out of 312) exhibited segregation distortion (for $P \leq 0.05$).

The power of detection of repulsion-phase linkages in polyploid species depends upon the mapping population size, the ploidy level and the chromosome-pairing behavior. Wu et al. (1992) have reported that when homologous chromosomes pair randomly (autopolyploids) the power of detection of repulsion-phase linkages is very low. In particular, with a population size of 67 and a confidence level of 5%, the maximum detectable distance in autotetraploids is 7.4 cM and no linkages can be detected at higher ploidy levels. In contrast, when chromosomes specifically choose their partners at meiosis, the detection-power of coupling and repulsion linkages is the same. As a consequence, if in a given pair of co-segregating groups the number of markers in coupling is comparable to the number of markers in repulsion, the chromosomes would pair preferentially. If, instead, the number of markers in coupling is much higher than the number of markers in repulsion, the chromosomes pair at random.

In highly polyploid species, such as sugarcane, some chromosomes pair preferentially (Grivet et al. 1996) and others at random, which suggests a mixed autopolyploid-allopolyploid origin. The pairwise analysis of co-segregational groups linked in repulsion suggests that at least some chromosomes pair preferentially in *P. pratensis*. Analysis of other *P. pratensis* genotypes would show whether this supposition is valid and applies to a wide range of genotypes.

For most co-segregational groups, a comparable number of co-segregating markers linked in repulsion was not identified, but we cannot rule out the possibility that some co-segregational groups were unique (with only one homologous group) or too small for a given homology group. Repulsion-phase linkage is fundamental for assembling co-segregational groups into homology groups. However, the construction of composite maps of each homology group requires large segregating populations, as well as a perfect knowledge of both pairing behavior and ploidy level.

We attempted to reconstruct a composite map of only those homology groups that linkage analysis indicated as pairing-preferentially. Because the addition of repulsion-phase markers did not change the genetic distances between markers in the composite map, the integration of the co-segregating groups was consistent. This approach could, therefore, prove useful for preliminary mapping analysis in highly polyploid species for which information about the chromosome pairing behavior are unavailable. Even though it is impossible to calculate the genetic distance between markers in repulsion with an acceptable degree of confidence, allowing for autoexaploids and with a population size of 75, it should be possible to identify co-segregating groups as candidates for preferential pairing by finding markers mutually linked in repulsion.

Objections may be raised about the convenience of using dominant molecular markers such as AFLPs and SAMPLs for constructing genetic maps in autopolyploids. In fact, unlike RFLPs and SSRs, which identify co-segregational groups on the basis of allellism (markers detected by the same probe or primer pair), they have the obvious disadvantage of making it difficult to identify homologous co-segregational groups. This disadvantage of AFLPs and SAMPLs is, however, partially offset by the higher yield of chromosome-specific markers. In fact

these markers may easily be converted to SCARs, which are very effective and useful for marker-assisted-selection applications.

Ongoing work is aimed to integrate the genetic map here presented with co-dominant molecular markers (SSRs). A detailed linkage map and suitable DNA markers for polyploid *P. pratensis* could definitely address basic questions such as the genetic control and regulation of apomixis, and lead to the cloning of apomixis-related genes.

Acknowledgements The authors thanks Dr. L. Molinari and Dr. S. Ancillotti for invaluable technical assistance, Prof. S. Tavoletti for helpful discussions, and Prof. O. Tanzarella and Dr. D. Rosellini for critical reading of this manuscript. This work is part of the project "Sistemi riproduttivi negli organismi di interesse agrario: applicazioni biotecnologiche in *Poa pratensis* e *Saccharomyces cerevisiae*" funded by the University of Perugia.

References

Averett JE (1980) Polyploidy in plant taxa. In: Lewis WH (ed) Polyploidy, biological relevance. Basic Life Science, New York, vol 13, pp 151–269

Barcaccia G, Mazzucato A, Albertini E, Zethof J, Gerats T, Pezzotti M, Falcinelli M (1998) Inheritance of parthenogenesis in *Poa pratensis* L.: auxin test and AFLP linkage analysis support monogenic control. Theor Appl Genet 97:74–82

Bashaw EC, Funk RC (1987) Apomictic grasses. In: Fehr WR (ed) Principles of cultivar development, vol 2. MacMillan, New York, pp 40–82

Clausen J (1961) Introgression facilitated by apomixis in polyploid *Poas*. Euphytica 10:87–94

Cnops G, Den Boer B, Gerats A, Van Montagu M, Van Lijsebettens M (1996) Chromosome landing at the *Arabidopsis Tornado1* locus using an AFLP-based strategy. Mol Gen Genet 253:32–41

D'Amato F (1995) Aneusomaty in vivo and in vitro in higher plants. Caryologia 44:85–103

Darlington CD, Wylie AP (1961) Chromosome atlas of flowering plants, George Allen and Unwin LTD, London

D'Hont A, Lu YH, Gonzales de Leon D, Grivet L, Feldmann P, Lanaud C, Glaszmann JC (1994) A molecular approach to unraveling the genetics of sugarcane, a complex polyploid of the Andropogoneae tribe. Genome 37:222–230

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Grivet L, D'Hont HA, Roques D, Feldmann P, Lanaud C, Glaszmann JC (1996) RFLP mapping in cultivated sugarcane (Saccharum spp.): genome organization in highly polyploid and aneuploid interspecific hybrids. Genetics 142:987–2000

Glaszmann JC, Fautret A, Noyer L, Feldmann P, Lanaud C (1989) Biochemical genetic markers in sugarcane.Theor Appl Genet 78:537–543

Glaszmann JC, Dufur P, Grivet L, D'Hont A, Deu M, Paulet F, Hamon P (1997) Comparative genome analysis between several tropical grasses. Euphytica 96:13–21

Hackett CA, Bradshaw JE, Meyer RC, McNicol JW, Milbourne D, Waugh R (1998) Linkage analysis in tetraploid species: a simulation study. Genet Res 71:144–154

Jacobs JME, van Eck HJ, Arens P, Verker-Bakker B, Hekkert BL, Bastiaanssen HJM, El-Kharbotly A, Pereira A, Jacobsen E, Stiekema WJ (1995) A genetic map of potato (Solanum tuberosum) integrating molecular markers, including trasposons and classical markers. Theor Appl Genet 91:289–300

Kiss GB, Csanadi G, Kalman K, Kalo P, Okresz L (1993) Construction of a basic genetic map for alfalfa using RFLP, RAPD, isozyme and morphological markers. Mol Gen Genet 90:1119–1127

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

Love A, Love D (1975) Cytotaxonomical atlas of artic flora, Leytershansen, Germany

Mather K (1957) The measurement of linkage in heredity. Methuen, London

Matzk F (1991) New efforts to overcome apomixis in *Poa pratensis* L. Euphytica 55:65–72

Mazzucato A (1995) Italian germplasm of *Poa pratensis* L. II. Isozyme progeny test to characterize genotypes for their mode of reproduction. J Genet Breed 49:119–126

Meyer WA, Funk CR (1989) Progress and benefits to humanity from breeding cool-season grasses for turf. In: Sleeper DA, Asay KH, Pedersen JF (eds) Contributions from breeding forage and turf grasses. CSSA Special Publ no. 15, pp 31–48

Morgante M, Vogel J (1994) Compound microsatellite primers for the detection of genetic polymorphisms. U.S. Patent Application No. 08/326456

Ripol MI, Churchill GA, da Silva JA, Sorrells M (1999) Statistical aspects of genetic mapping in autopolyploids. Gene 235:31–41

Speckmann GJ, van Dijk GE (1972) Chromosome numbers and plant morphology in some ecotypes of *Poa pratensis* L. Euphytica 21:171–180

Stam P, Van Ooijen JW (1995) JoinMap version 2.0: software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen, The Netherlands

Sorrels ME (1992) Development and application of RFLPs in polyploids. Crop Sci 32:1086–1091

Tavoletti S, Veronesi F, Osborn TC (1996) RFLP linkage map in an alfalfa diploid mutant based on F_1 populations. J Hered 87:167–170

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

Wedin WF, Huff DR (1996) Bluegrasses. In: Moser LE, Buxton DR, Casler MD (eds) Cool-season forage grasses, American Society of Agronomy, Madison, Wisconsin, USA, vol 34, pp 665–690

Wu KK, Burnquist W, Sorrells ME, Tew TL, Moore PH, Tanksley SD (1992) The detection and estimation of linkage in polyploids using single-dose restriction fragments. Theor Appl Genet 83:294–300

Zabeau M, Vos P (1992) European patent application. Publication no. 0543 858 Al